Oxidation of 4-(3-Indolyl)- and 4-(5-Indolyl)-1,4-dihydropyridines in Aprotic and Protic Media: Reactivity toward Alkylperoxyl Radicals

R. Salazar,[a] P. A. Navarrete-Encina,[b] C. Camargo,[c] J. A. Squella,[a,*] and Luis J. Núñez-Vergara[a,*,z]

[a]Laboratory of Biolectrochemistry, [b]Laboratory of Organic Synthesis and Molecular Modeling, and [c]Laboratory of Antidoping, Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile

Electrochemical oxidation of 4-(3-indolyl)- and 4-(5-indolyl)-1,4-dihydropyridines (DHPs) in aprotic and protic media is reported. Also, the reactivity of compounds toward alkylperoxyl radicals 2,2'-azobis-(2-amidinopropane) dihydrochloride-derived in aqueous media at pH 7.4 is assessed. Derivatives were electrochemically oxidized exhibiting two anodic signals in both electrolytic media. The first signal is due to oxidation of the dihydropyridine ring, and the second one is due to oxidation of the indolyl moiety. Electron spin resonance experiments proved the formation of carbon-centered dihydropyridyl radicals as intermediates in the oxidation of the dihydropyridine moiety in aprotic medium. Pyridine was identified as the final product of the oxidation in both electrolytic media by gas chromatography/mass spectrometry. The 4-substituted 1,4-DHPs were more reactive than tested commercial 1,4-DHPs toward alkylperoxyl radicals.

The 4-substituted Hantzsch dihydropyridines, analogs of NADH coenzymes, are an important class of drugs. The 1,4-dihydropyridines (DHPs) are allosteric modulators that act on L-type Ca^{2+} channels in a voltage-dependent manner either as antagonists or agonists.[1] The former is clinically used for the treatment of cardiovascular diseases, including hypertension.[2,3] In the human body, these compounds undergo oxidation to form pyridine derivatives. These oxidized compounds are largely devoid of the pharmacological activity, when compared to their parent compounds.

To reach more selective and long-acting drugs with fewer side effects, structural modifications have been made. Changes in the substituent pattern of the C-3, C-4, and C-5 positions of the dihydropyridine ring alter activity and tissue selectivity.[4,5]

Our laboratory has been working on the synthesis and electrochemical characterization of DHPs for several years. This effort has focused on the synthesis of derivatives with stronger antioxidant properties and the study of the interactions of different redox centers coexisting in the same molecule. We considered the indole as a good choice for a second redox center because it has been reported to possess a wide variety of biological properties, including anti-inflammatory, antibacterial, anticonvulsant, and antioxidant properties.[6-8] Consistent with the above-mentioned facts, in this article the synthesis and electrochemical characterization of two compounds [4-(3-indolyl)-DHP and 4-(5-indolyl)-DHP], both having a dihydropyridine ring and an indole ring acting as redox centers in the same molecule, are studied. Lavilla et al.[9] have previously reported the design, synthesis, and pharmacological evaluation of a series of 4-(3-indolyl)dihydropyridines. The tested DHPs showed the same potency as nifedipine but lower efficacy in blocking the KCl-contractions in rat aorta and vas deferens (part of the male reproductive system in humans). The inhibited production of radical oxygen-derived species was also similar to that of nifedipine, being related to the antioxidant properties of dihydropyridines, regardless of their substitution pattern.

From the electrochemical point of view, the study of the oxidation of some 1,4-DHP has previously been performed preferentially in nonaqueous media using mainly a rotating ring-disk electrode, linear and cyclic voltammetry (CV), and controlled-potential electrolysis coupled with electron spin resonance (ESR) spectroscopy.[10-14] In this framework, the electrochemical oxidation of a series of new DHPs in aprotic and protic media has been also reported by our group.[15-19]

In addition, in this paper the electrochemical oxidation in both aprotic and protic media of two synthesized and characterized 4-indolyl 1,4-dihydropyridines is reported. Results are compared to the 4-phenyl substituted 1,4-dihydropyridine derivative. The reactivity of compounds toward alkylperoxyl radicals 2,2'-azobis-(2-amidinopropane) dihydrochloride (ABAP)-derived at pH 7.4 is also assessed.

Experimental

C4-indolyl substituted 1,4-dihydropyridines (Fig. 1).— A mixture of 6 mmol of indole 3-carboxaldehyde or indole 5-carboxyaldehyde in 20 mL of ethyl alcohol was mixed with 0.015 mol of ethyl acetoacetate and 0.01 mol of concentrated ammonium hydroxide. This mixture was heated under reflux for 30 h

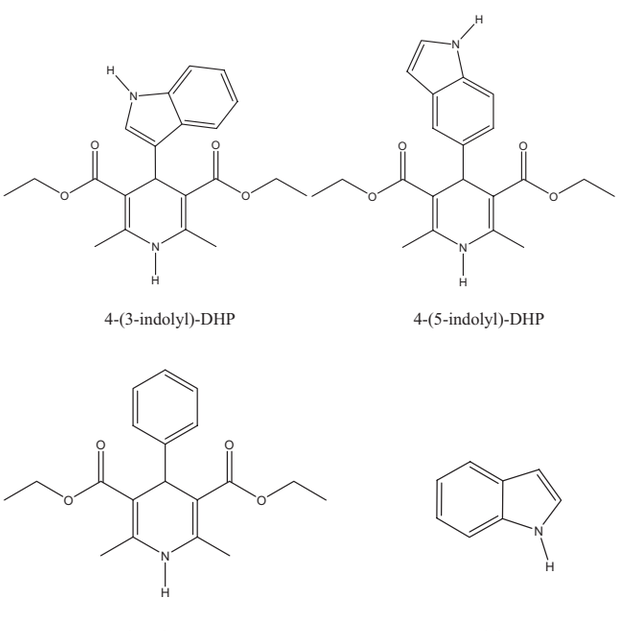

Figure 1. Chemical structures of 4-(3-indolyl)-DHP, 4-(5-Indolyl)-1,4-DHP, 4-phenyl-DHP, and indole.

* Electrochemical Society Active Member.
z E-mail: lnunezv@ciq.uchile.cl

under nitrogen. The crude solid obtained was filtered and recrystallized from ethyl alcohol/water (50/50). Synthesized compounds had the following characteristics.

2, 6-dimethyl-3, 5-diethoxycarbonyl-4-(3-indolyl)-1, 4-dihydropyridine [4-(3-indolyl)-DHP].— Yield: 73%. mp.:183–184°C. IR (KBr): 3344.4; 2978.1; 1676.7; 1487.1; 1367.9; 1305.6; 1215.4; 1100.3; 1020.4; 807.2; 744.7 cm^{-1}. ^{1}H-NMR (300 MHz, dimethyl sulfoxide (DMSO)-d_6): δ_{max} 1.18 (t, 6H, 2x $-CH_2CH_3$); 2.31 (s, 6H, 2x $-CH_3$); 4.03 (q, 4H, 2x $-OCH_2CH_3$); 5.23 (s, 1H, ArCH); 6.97 (m, 3H, J = 7.83 Hz, 3x ArH); 7.33 (d, 2H, J = 7.92 Hz, 2x ArH); 7.92 (d, 2H, J = 7.92 Hz, 2x ArH); 8.89 (s, 1H, DHP-NH); 10.74 (s, 1H, indole-NH). ^{13}CNMR (75 MHz, DMSO-d_6): 13.70; 17.64: 29.98; 58.25; 101.43; 110.85; 117.54; 118.91; 119.77; 121.31; 122.17; 125.25; 135.54; 143.58; 166.71. Anal. Calcd. for $C_{21}H_{24}N_2O_4$: C 68.46; H 6.57; N 7.60. Found: C 68.16; H 6.55; N 7.61.

2, 6-dimethyl-3, 5-diethoxycarbonyl-4-(5-indolyl)-1, 4-dihydropyridine [4-(5-indolyl)-DHP].— Yield: 61%. mp.:178–180°C. IR (KBr): 3351.2; 2978.5; 1657.6; 1481.6; 1371.4; 1303.5; 1208.2; 1100.5; 1018.2; 727.0 cm^{-1}. ^{1}HNMR (300 MHz, DMSO-d_6): δ 1.20 (t, 6H, 2x $-CH_2CH_3$); 2.32 (s, 6H, 2x $-CH_3$); 4.04 (q, 4H, 2x $-OCH_2CH_3$); 4.99 (s, 1H, ArCH); 6.37 (s, 1H, ArH); 7.00 (d, 1H, J = 7.92 Hz, 1x ArH); 7.25 (m, 3H, J = 7.56 Hz, 3x ArH); 8.76 (s, 1H, DHP-NH); 10.94 (s, 1H, indole-NH). ^{13}CNMR (75 MHz, DMSO-d_6): 13.74; 17.96: 48.802; 58.37; 100.20; 102.53; 110.01; 117.72; 120.76; 124.49; 127.07; 134.08; 138.52; 143.88; 166.71. Anal. Calcd. for $C_{21}H_{24}N_2O_4$: C 68.46; H 6.57; N 7.60. Found: C 68.70; H 6.60; N 7.58.

2,6-dimethyl-3,5-diethoxycarbonyl-4-phenyl-1,4-dihydropyridine (4-phenyl-DHP) was synthesized according to a previous paper 6.— Yield: 92%. mp.:150–153°C. ^{1}HNMR (300 MHz, DMSO-d_6): δ (1.16 (t, 6H, 2x $-CH_2CH_3$); 2.26 (s, 6H, 2x $-CH_3$); 3.98 (q, 4H, 2x $-OCH_2CH_3$); 4.88 (s, 1H, ArCH); 7.18 (m, 3H, J = 6.975 Hz, 3x ArH); 7.22 (d, 2H, J = 8, 14 Hz, 2x ArH); 8.80 (s, 1H, NH). ^{13}CNMR (75 MHz, DMSO-d_6): 10.51; 9.26; 9.07; 7.88; 7.78; 6.18; 2.09; 2.05; 2.04; 1.97; 0.63. Anal. Calcd. for $C_{19}H_{23}O_4N$: C:62.28; H: 7.04; N: 4.25. Found: C: 62.08; H: 6.98; N: 4.30.

Electrolytic media.— Aprotic medium: Dimethyl sulfoxide (DMSO) containing 0.1 M tetrabutylammonium hexafluorophosphate (TBAPF6). Protic medium consisted of 0.04 M Britton–Robinson buffer/ethanol: 70/30 and adjusted to an ionic strength of 0.1 M with KCl.

Voltammetry.— Differential pulse (DPV) and CV were performed with a bioanalytical system (BAS)-CV 100 assembly. All voltammetric experiments were carried out with 1 mM solutions of DHPs. A stationary glassy carbon electrode was used as the working electrode (0.07 cm^2) for DPV and CV experiments. For hydrodynamic experiments, a rotating disk glassy carbon electrode (0.07 cm^2) was employed. The surface of the electrode was polished to a mirror finish with alumina powder (0.3 and 0.05 μm) before use and after each measurement. Platinum wire was used as an auxiliary electrode, and all potentials were measured against an Ag/AgCl electrode in aqueous saturated KCl.

Controlled-potential electrolysis (CPE).— Exhaustive electrolysis at a reticulated glassy carbon electrode was carried out at the following potentials: Aprotic medium: 1100 and 1500 mV. Protic medium: 900 and 1300 mV at pH 3. At pH 11, the applied potentials were 550 and 700 mV. A BAS-CV 100 assembly was used to electrolyze the 1,4-DHPs solutions. Net charge was calculated, including correction for the background current.

UV/vis spectrometry.— UV/visible (UV/vis) spectra were recorded in the 200–1000 nm range by using an Agilent spectrophotometer with a diode array using 1 cm quartz cell.

ESR.— Spectra were recorded in situ on a Bruker spectrometer ECS 106 with 100 kHz field modulation in X band (9.78 GHz) at room temperature. The hyperfine splitting constants were estimated to be accurate within 0.05 G. Electrolysis was performed in the ESR cell using an appropriate platinum mesh electrode according to the same conditions as described above by using 1 mM 1,4-DHP solutions in the presence of 100 mM N-*tert*-butylamine-α-phenylnitrone (PBN), which was used as spin trap.

GC-MS.— A gas chromatography/mass spectrometry (GC-MS) detector (5890/5972) combination (Hewlett-Packard, Palo Alto, CA, USA) and a Hewlett-Packard 7673 autosampler were used for the analyses. The m/z range monitored was 45–550 with a scan rate of 1 scan/s; the normal electron energy was 70 eV. A Hewlett-Packard Ultra-1 column, 25 cm–0.2 mm id–0.11 mm film thickness (Little Falls, Wilmington, DE, USA), was used.

Reactivity toward alkylperoxyl ABAP-derived radicals.— ABAP [2,2'-azobis-(2-amidinopropane) dihydrochloride]. Different series of 100 mM ABAP solutions in 0.04 M Britton–Robinson buffer/*N*,*N*-dimethylformamide (DMF) 70/30 pH 7.4 at a constant ionic strength of 0.1 M adjusted with KCl were incubated with different solutions (20–200 μM) of 1,4-DHP synthesized and commercial 1,4-DHPs at 37°C for 120 min with constant bubbling of oxygen. From these experiments, reaction rates were calculated for each 1,4-DHP derivative. The rate of alkylperoxyl radical formation from ABAP is constant at a given temperature.[20,21] However, the rate of formation of alkylperoxyl radicals from this initiator will not be constant because it depends on the concentration of ABAP (rate = k[ABAP]). It appears that over 120 min at 37°C, only a small amount of the ABAP will decay; therefore, the rate may be considered constant at 37°C. In neutral aqueous solutions, the half-life of ABAP is about ~175 h, and the generation rate of radicals is constant for the first few hours.[20,21] Control solutions containing either DHP solution were run under the same conditions as the above mixtures. The time course of the reactivity of synthesized 1,4-DHP derivatives with the generated alkylperoxyl radicals was followed by UV/vis spectroscopy and GC-MS techniques.

According to the previous text, we can assume that the high [ABAP] decomposes slowly at 37°C, but it maintains [$ROO^{\cdot}$] at least 25-fold greater than [DHP]'s to ensure pseudo first-order conditions.

As can be seen later, in Fig. 7, plots of ln [DHP] vs time are linear with a slope value equal to k'. The linearity of the plots [r = 0.9997 for 4-(3-indolyl)-DHP and r = 0.9994 for 4-(5-indolyl)-DHP] supports the original assumption of pseudo first-order kinetics for the reaction. Rate constants for 1,4-DHP were calculated from five independent experiments. Also the reactivity toward alkylperoxyl radicals was compared to commercial 1,4-DHPs by using the following ratio (r)

$$r = k \text{ value commercial 1,4-DHP}/k \text{ value DHP tested}$$

Control solutions (in the absence of ABAP-derived radicals) revealed no changes either in their original UV/vis absorption bands or GC-MS fragmentation. Also, a possible photodecomposition of 1,4-dihydropyridines was assessed, but in the time scale of the experiments this was negligible.

Results

Aprotic medium.— The main goal of the present work was the study of the oxidation of two synthesized 4-indolyl-1,4-dihydropyridines to investigate the possible reciprocal interactions between two redox centers coexisting in the molecule (i.e., the 1,4-dihydropyridine ring and the indole moiety).

In Fig. 2A, differential pulse (DP) voltammograms on a glassy carbon electrode for the different derivatives are shown. As can be seen, 4-indolyl-DHP derivatives exhibits two well-defined oxidation signals. A first oxidation signal was found between 944 and 992 mV, for 4-(3-indolyl)-DHP and 4-(5-indolyl)-DHP, respectively.