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ELECTROCHEMICAL OXIDATION OF COMPOUNDS CONTAINING 1,4-DIHYDRO-PYRIDINE AND PYRIDINIUM RINGS – ANALOGS OF GENE TRANSFECTION AGENTS

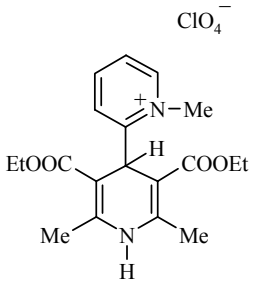
B. Turovska, J. Stradins, I. Turovskis, A. Plotniece, A. Shmidlers, and G. Duburs

A study was carried out on the electrochemical oxidation of 1,4-dihydropyridines, found as substituents in pyridinium salts, which are strong electron acceptors. The potentials for their oxidation in acetonitrile were determined. NMR spectroscopy was used to find the relative acidity of the N–H and C–H protons and the oxidation potentials were determined for the anionic products of the ionization of the N–H bond in dihydropyridine. The only product of the preparative electrolysis, in contrast to chemical oxidation, is the corresponding pyridine, namely, the oxidized dihydropyridine form.

Keywords: dihydropyridine anions, dipyridines, 1,4-dihydropyridines, oxidation mechanism, electrochemical oxidation.

Dihydropyridines, which have a broad spectrum of biological activity [1], including acting as gene transfection agents [2], have been the subject of extensive investigation [3, 4]. In an electrochemical study of the individual steps involving transfer of electrons (E) and protons (C) in the dihydropyridine–pyridine transformation, all possible mechanisms, namely, EC, EECC, and ECEC, have been examined. Such a variety of results can be explained only if we assume the strong dependence of the oxidation mechanism on the structure of the dihydropyridines studied as well as the experimental conditions.

In the present work, we studied the electrochemical oxidation of the 1,4-dihydropyridine ring at $C_{(2)}$ (**1**), $C_{(3)}$ (**2**), and $C_{(4)}$ (**3**) of a pyridinium salt by cyclic voltammetry and preparative electrolysis at constant potential.



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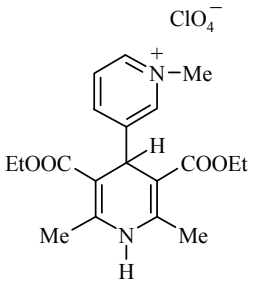

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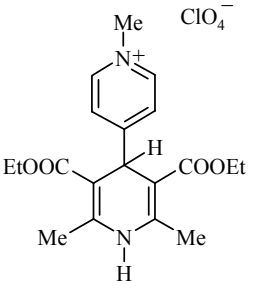

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Latvian Institute of Organic Synthesis, Riga LV-1006, Latvia; e-mail: turovska@osi.lv. Translated from Khimiya Geterotsiklicheskikh Soedinenii, No. 6, pp. 880-886, June, 2004. Original article submitted April 14, 2004.

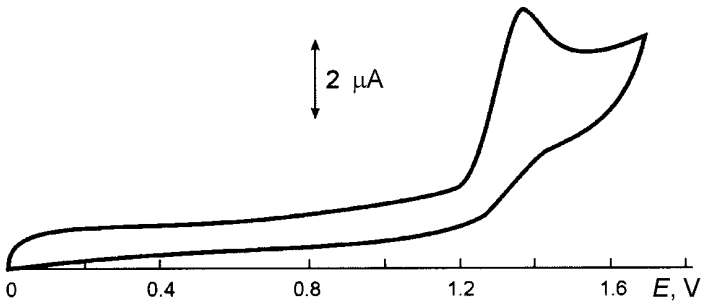
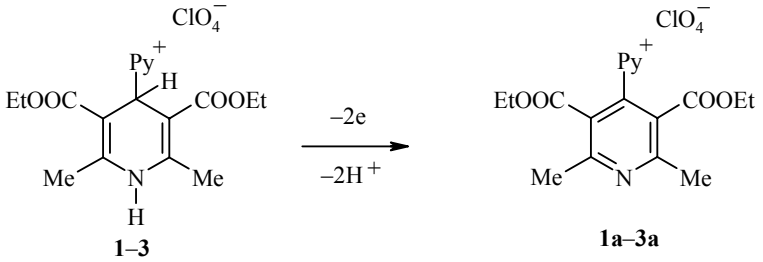


Fig. 1. Electrochemical oxidation of **1** ($c = 5\times10^{-4}$ M) in 0.1 M $NaClO_4$ in acetonitrile on a platinum electrode.

The oxidation of **1-3** in acetonitrile proceeds in one irreversible step (Fig. 1) at the potentials indicated in Table 1. As expected. the oxidation of the dihydropyridine ring is most difficult when it is located at $C_{(2)}$ in the pyridinium ring. Dihydropyridines found at $C_{(3)}$ and $C_{(4)}$ of the pyridinium ring are more readily oxidized by 200 mV. Coulometric measurements taken during preparative oxidation (Table 1) indicate the two-electron nature of the anodic process. The presence of traces of oxygen in the solution reduces the number of electrons participating in the electrode process to a value close to 1*e* due to the parallel oxidation of the formed radical–cations by oxygen. In the chemical oxidation of 1,2',6'-trimethyl-3',5'-bis(ethoxycarbonyl)-1',4'-dihydro-3,4'-bipyridinium iodide [1] by sodium nitrite or hydrogen peroxide tar formation occurs. Thus, the corresponding oxidation product was synthesized independently by the oxidation of a derivative of 1',4'-dihydro-3,4'-bipyridine with subsequent alkylation of the dipyridine obtained [1]. In contrast to the chemical oxidation, **1a-3a** are the only products of the preparative electrolysis.

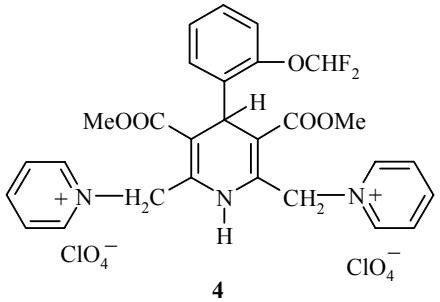


1–3 **1a–3a**

TABLE 1. Oxidation Potentials (E^{ox}) of **1-4** and **6**, Their Anions (E^{ox}_{an}) and Number of Electrons (*n*) Participating in Preparative Oxidation

Compound	E^{ox}, V	E^{ox}_{an}, V	*n*
1	1.50	0.30	1.70
2	1.27	0.24	2.00
3	1.27	0.29	1.75
4	1.55	0.50	1.71
6	0.90	—	1.80

The anodic oxidation of the 1,4-dihydropyridine fragment in bispyridinium salt **4** process more anodicaly than the analogous oxidation in **1-3**. The potential of the peak reaches 1.55 V and the process is irreversible (Fig. 2).



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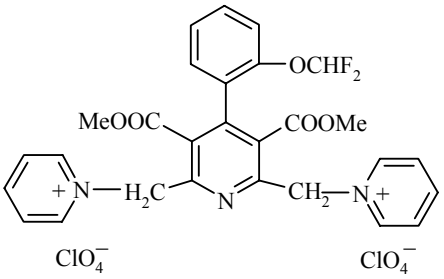
The preparative electrolysis at 1.6 V is a two-electron process in the absence of oxygen. The structure of the only oxidation product **4a** was indicated by ^{1}H NMR spectroscopy.



4a

As the electrochemical oxidation of **1-4** is a two-electron process, it may thereby correspond to the ECEC or EECC mechanism only. Since both the N–H and C–H protons in the dihydropyridine fragment are involved in the oxidation, we should clarify which of these protons is more acidic. For this purpose, various

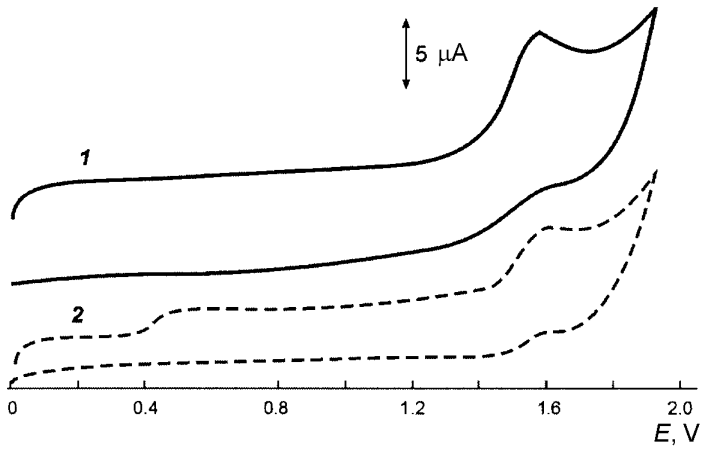


Fig. 2. Electrochemical oxidation of **4** ($c = 5\times10^{-4}$ M) in 0.1 M $NaClO_4$ in acetonitrile on a platinum electrode (**1**) and in the presence of Na_2CO_3 (**2**).

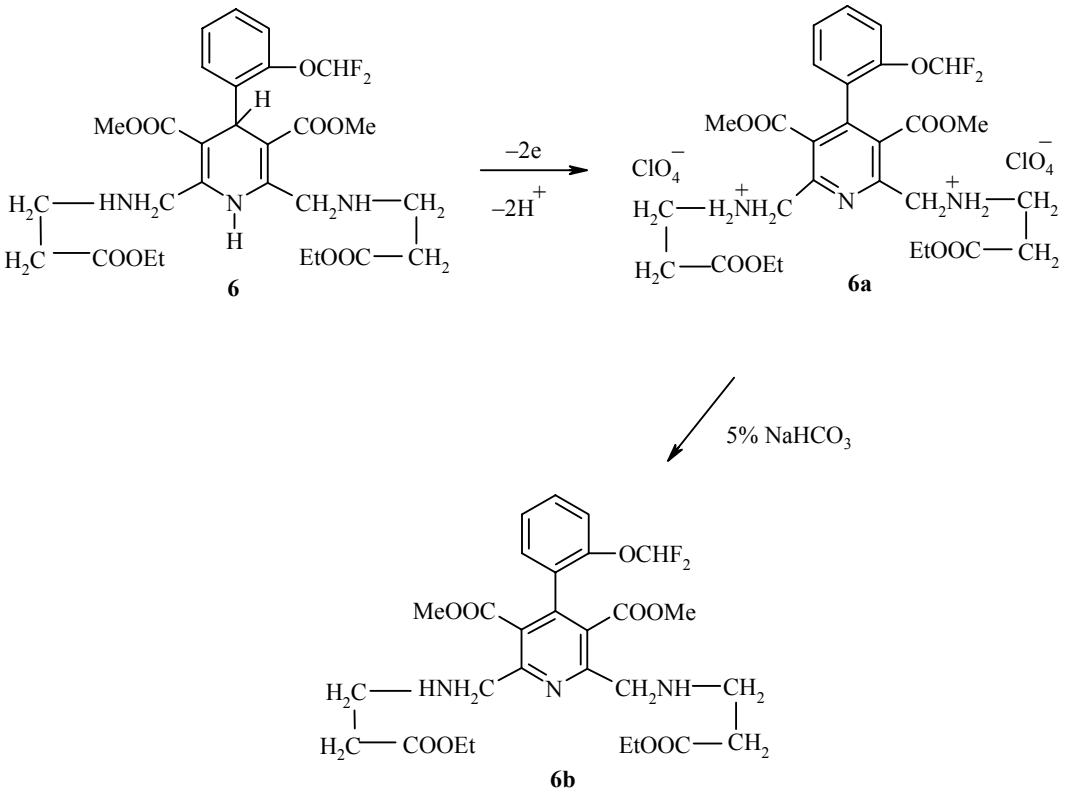
bases were added to solutions of the compounds studied. Thus, the addition of Na_2CO_3 to a solution of **4** in DMSO-d_6 leads to formation of dark red anion **5**, while the N–H signal disappears from the ^{1}H NMR spectrum. According to our previous study [2], for analogs of **4** containing decyl, dodecyl, tetradecyl, and hexadecyl residues the values of pK_a is close to 7.

A stronger base such as potassium *tert*-butoxide is required to obtain the anions of **1-3**. Despite the geminal arrangement of a strong electron acceptor and a proton at C-4', the loss of the N–H signal is the only change in the ^{1}H NMR spectrum in the presence of potassium *tert*-butoxide. Thus, the N–H proton is the most acidic in all **1-4**, regardless of the position of the pyridinium residue or residues. The acidity of the N–H bond and pK_a value for the series of Hantzch 1,4-dihydropyridines were determined by Vigante et al. [5]. Upon oxidation, the acidity of the primary products (radical–cations) are several orders of magnitude greater than the acidity of the starting compounds [6, 7]. Thus, the rapid loss of the N–H proton must follow the removal of the first electron from the nitrogen atom in the dihydropyridine. Hence, **1-4** are oxidized according to the ECEC mechanism.

In the presence of Na_2CO_3, an additional peak appears on the cyclic voltammograms of **4** at +0.5 V, corresponding to oxidation of the dihydropyridine anion. Anions **1-3** are oxidized at 0.30, 0.24, and 0.29 V, respectively. The potentials of the electrochemical oxidation peaks of several 4-substituted Hantzch 1,4-dihydropyridines were determined by Lopez–Alarcon et al. [8], who found that these anions are oxidized at about 0 V more readily by 500 mV than the starting molecules.

The preparative electrolysis at the oxidation potentials of the anions of **1-4** could not be carried out to completion due to the rapid blockage of either the platinum or glassy carbon electrode by oxidation intermediates.

The electrochemical oxidation of dihydropyridine **6**, which has substituents capable of protonation at both $C_{(2)}$ and $C_{(6)}$, proceeds by the same ECEC mechanism. The only product of the two-electron irreversible oxidation at 0.9 V is pyridine **6a**.



A pyridine derivative with protonated substituents at $C_{(2)}$ and $C_{(6)}$ is obtained in the oxidation of dihydropyridine **6**, which is also a two-electron process. Therefore, **1-4** and **6** are oxidized through an ECEC mechanism, which is most frequently encountered in the literature for 1,4-dihydropyridines [8-10].

EXPERIMENTAL

The cyclic voltammetry and preparative electrolysis were carried out in a PAR-170 electrochemical system. The oxidation potentials were found on a stationary platinum electrode (d = 2 mm). All the potentials were measured relative to the saturated calomel electrode. A platinum wire served as the auxiliary electrode. The solvent was purified by adding $KMnO_4$ (2-3 g) to acetonitrile (2.5 l) and distill. Then, P_2O_5 (5 g) was added to the distillate, which was then redistilled. The acetonitrile sample was stored over CaH_2 (10 g) and distilled immediately prior to use. Anhydrous $NaClO_4$ dried in vacuum at 40°C was used as the supporting electrolyte.

The preparative oxidation was carried out in an H-shaped cell, bubbling argon through both the anodic and cathodic compartments during the entire electrolysis. The anode and cathode were both 4×2.8-cm platinum mesh screens. The electrolysis was carried out in 0.1 M $NaClO_4$ in acetonitrile (100 ml) at the oxidation potential of each compound. The cell was filled with a solution of the supporting electrolyte. The anodic section was loaded with **1-3** (1.00 g, 2.26 mmol), **4** (1.00 g, 1.39 mmol), or **5** (0.50 g, 0.84 mmol). After completion of the electrolysis, the reaction mixture was evaporated. In order to remove $NaClO_4$, the residue was subjected twice to chromatography on a 2×70-cm column packed with Acros 0.035-0.070-mm silica gel using 9:1 methylene chloride–methanol as the eluent.

The ^{1}H NMR spectra were taken on a Bruker WH-90 spectrometer at 90 MHz in DMSO-d_6 for **1-4** and chloroform-d for **5a** and **5b** with HMDS (δ 0.055 ppm) as the internal standard.

The perchlorates of **1-4** were obtained from the corresponding bromides [11, 12].

1,1'-{[3,5-Bis(methoxycarbonyl)-4-(2-difluoromethoxyphenyl)-1,4-dihydropyridine-2,6-diyl]-dimethylene}bispyridinium Diperchlorate (4). A sample of 1,1'-{[3,5-bis(methoxycarbonyl)-4-(2-difluoromethoxyphenyl)-1,4-dihydropyridine-2,6-diyl]dimethylene}bispyridinium dibromide (1.74 g, 2.55 mmol) [12] was dissolved in ethanol (60 ml) and, then, concentrated (57%) $HClO_4$ (8.7 ml) was added to the solution heated to reflux. The mixture was cooled to -10°C. The precipitate formed was filtered off and washed with two 5-ml ethanol portions and, then, two 5-ml ether portions to give 1.60 g of crystalline product. The crude product was redissolved in ethanol (300 ml) at reflux and 7.6 ml conc. $HClO_4$ was added to give 1.42 g (77%) colorless **4** as fine crystals; mp 199-208°C, R_f 0.15 (Silufol UV-254, 1:1 acetic acid–water). ^{1}H NMR spectrum, δ, ppm (J, Hz): 9.85 (1H, br. s, NH); 8.95 (4H, d, J = 6.0, H-2, H-2', H-6, H-6'); 8.60 (2H, t, J = H-4, H-4'); 8.10 (4H, dd, J = 7.0, J = 6.0, H-3, H-3', H-5, H-5'); 6.85-7.50 (4H, m, Ar); 7.13 (1H, t, J = 73.5, CHF_2); 5.70 (4H, AB q, J = 14.2, CH_2); 5.38 (1H, s, CH dihydropyridine); 3.55 (6H, s, CH_3). Found, %: C 46.59; H 3.66; N 5.67. $C_{28}H_{27}Cl_2F_2N_3O_{13}$. Calculated, %: C 46.55; H 3.77; N 5.82.

N-Deprotonated 5. ^{1}H NMR spectrum, δ, ppm, (J, Hz): 8.65 (4H, d, J = 6.0, H-2, H-2', H-6, H-6'); 8.51 (2H, t, J = 7.0, H-4,H-4'); 7.95 (4H, dd, J = 6.0, H-3, H-3', H-5, H-5'); 6.75-7.40 (4H, m, Ar); 7.03 (1H, t, J = 73.5, CHF_2); 5.65 (4H, AB q, J = 14.2, CH_2); 5.30 (1H, s, CH dihydropyridine); 3.46 (6H, s, CH_3).

Products 1-3 were obtained from the corresponding bromides [11, 12] by treating these bromides with equimolar amounts of conc. (57%) $HClO_4$ and then isolating perchlorates **1-3** according to the procedure for **4**.

Dimethyl Ester of 2,6-Bis{[(2-ethoxycarbonylethyl)amino]methyl}-4-(2-difluoromethoxyphenyl-1,4-dihydropyridine-3,5-dicarboxylic Acid (6) was prepared according to our previous reports [12, 13].

3',5'-Bis(ethoxycarbonyl)-1,2',6'-trimethyl-2,4'-bipyridinium perchlorate (1a). ^{1}H NMR spectrum, δ, ppm, (J, Hz): 9.25 (dd, J = 6.0, J = 1.0, H-3); 8.65 (1H, td, J = 7.4, J = 1.0, H-5); 8.22 (1H, ddd, J = 7.4, J = 6.0, J = 1.0, H-4); 8.08 (1H, dd, J = 7.4, J = 1.0, H-6); 4.05 (3H, s, NCH_3); 4.01 (4H, q, J = 7.0, $\underline{CH_2}CH_3$); 2.71 (6H, s, CH_3); 0.94 (6H, t, J = 7.0, $CH_2\underline{CH_3}$).

3',5'-Bis(ethoxycarbonyl)-1,2',6'-trimethyl-3,4'-bipyridinium Perchlorate (2a). ^{1}H NMR spectrum, δ, ppm, (*J*, Hz): 9.18 (1H, s, H-2); 9.08 (1H, d, *J* = 6.0, H-4); 8.59 (1H, d, *J* = 8.0, H-6); 8.23 (1H, dd, *J* = 8.0, *J* = 6.0, H-5); 4.40 (3H, s, NCH_3); 4.06 (4H, q, *J* = 7.0, $\underline{CH_2}CH_3$); 2.61 (6H, s, CH_3); 0.97 (6H, t, $CH_2\underline{CH_3}$).

3',5'-Bis(ethoxycarbonyl)-1,2',6'-trimethyl-4,4'-bipyridinium perchlorate (3a). ^{1}H NMR spectrum, δ, ppm, (*J*, Hz): 9.01 (2H, d, *J* = 6.0, H-3, H-5); 8.01 (2H, d, *J* = 6.0, H-2, H-6); 4.41 (3H, s, NCH_3); 4.05 (4H, q, *J* = 7.0, $\underline{CH_2}CH_3$); 2.58 (6H, s, CH_3); 1.01 (6H, t, *J* = 7.0, $CH_2\underline{CH_3}$).

1,1'-{[3,5-Bis(methoxycarbonyl)-4-(2-difluoromethoxyphenyl)pyridine-2,6-diyl]dimethylene}bis-pyridinium Diperchlorate (4a). ^{1}H NMR spectrum, δ, ppm, (*J*, Hz): 8.71 (4H, d, *J* = 6.0, H-2, H-2', H-6'); 8.60 (2H, t, *J* = 7.5, H-4, H-4'); 8.00 (4H, dd, *J* = 7.5, *J* = 6.0, H-3, H-3', H-5, H-5'); 7.0-7.7 (4H, m, Ar); 7.27 (1H, t, 73.5, CHF_2); 6.15 (4H, s, CH_2); 3.57 (6H, s, CH_3).

N,N'-{[3,5-Bis(methoxycarbonyl)-4-(2-difluoromethoxyphenyl)pyridine-2,6-diyl]dimethylene}-N,N'-bis(2-ethoxycarbonylethyl)diammonium Diperchlorate (6a). ^{1}H NMR spectrum, δ, ppm, (*J*, Hz): 7.25 (4H, m, Ar); 6.81 (4H, br. s, N^+H_2); 6.37 (1H, t, *J* = 73.0, CHF_2); 4.72 (4H, s, CH_2); 4.19 (4H, q, *J* = $\underline{CH_2}CH_3$); 3.59 (4H, t, *J* = 6.0, NCH_2); 3.52 (6H, s, CH_3); 2.97 (4H, t, *J* = 6.0, $COCH_2$); 1.27 (6H, t, *J* = 7.0, $CH_2\underline{CH_3}$).

Dimethyl Ester of 2,6-Bis{[(2-ethoxycarbonyl)amino]methyl}-4-(2-difluromethoxyphenyl)-pyridine-3,5-dicarboxylic Acid (6b). ^{1}H NMR spectrum, δ, ppm, (*J*, Hz): 7.12 (4H, m, Ar); 6.36 (1H, t, *J* = 74.0, CHF_2); 4.10 (4H, q, *J* = 7.01, CH_2CH_3); 3.97 (4H, s, CH_2); 3.49 (6H, s, CH_3); 2.87 (4H, t, *J* = 7.0, NCH_2); 2.48 (4H, t, *J* = $COCH_2$); 2.20 (2H, br. s, NH); 1.22 (6H, t, *J* = 7.0, CH_2CH_3).

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