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Interdependent chemical-electrochemical steps in retrometabolism-based drug and safer chemical design

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An extension of the retrometabolic based drug (chemical) design concept, specifically the soft drug approach, to the family of nitrone compounds is presented. Nitrones oppose oxidative challenges by virtue of their ability to very rapidly trap free radical species that are more stable and biochemically less harmful than the original molecular fragments. Moreover, the spin adducts may undergo further transformations including reaction with a second radical and decomposition (hydrolysis) to hydroxylamines and carbonyl compounds. Nitrones and their spin adducts may generate nitric oxide *in vivo*, which, like nitrones themselves, exerts a number of diverse activities in phylogenetically distant species as well as opposing effects in related biological systems. It was described as a major messenger in the cardiovascular, immune, and nervous systems, in which it plays regulatory, signaling, cytoprotective, and cytotoxic effects. Nitrones play an important role in the synthesis of drugs belonging to chemically and pharmacologically very different classes. A combined chemical-electrochemical synthesis of nitrones has been elaborated. These compounds may be obtained from aldehydes or ketones and N-substituted hydroxylamines. These reactions were performed directly, in situ in the electrochemical cell, where phenylhydroxylamine obtained by electroreduction of nitrobenzene derivatives reacts with the carbonyl compound introduced in the cell. The kinetic and thermodynamic parameters of the processes were determined by analyzing the adequate polarographic curves. Differences between purely chemical and mixed chemical-electrochemical methods are discussed. Analysis of the experimental data permits optimization of the investigated process from a preparative point of view. Effects of structural factors were systematically evaluated. The proposed method may be useful for combinatorial chemistry as well.

1. Introduction

Under properly selected conditions, different functional groups can be introduced in the structure of a studied lead component directly in the electrochemical cell. In this way, electrochemically active C–C, C–O, C–N, C–X (halogen) and other bonds are transformed into the desired product by consecutive electrochemical-chemical reactions [1]. Similar reactions may be utilized to synthesize soft drugs and soft chemicals, too. Aromatic nitro-derivatives are very instructive from this point of view. Cathodic reduction of these compounds is frequently complicated by diverse chemical processes (condensations, rearrangements) that take place between certain of their intermediate products (azomethines etc.). The exact nature of the component chemical-electrochemical steps is a function of the experimental conditions (pH, temperature, ionic strength etc.). Consequently, the reaction course can be directed by addition of different substances.

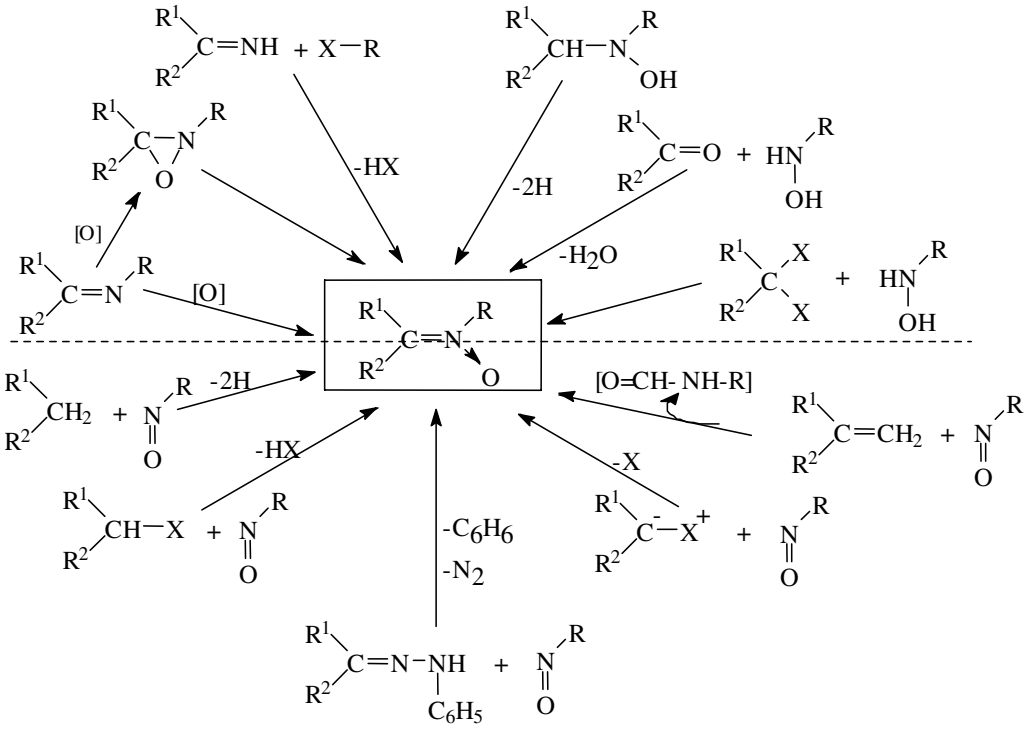
We have studied the electrochemical-chemical synthesis of nitrones (oxyazomethines). During the last decades, this compound family has been investigated very extensively because of its importance in chemistry, pharmacy, and biology. Chlordiazepoxide, the first tranquilizer of the benzodiazepine series introduced in therapy (1960), contains a nitrone moiety. This functional group was incorporated in initial combinations or intermediate products used for the synthesis of numerous biologically active substances since over 80 years ago (e.g., ephedrine and its derivatives) [2–6]. Currently, a great variety of nitrones are employed in different steps of the synthesis of drugs and chemicals, embracing a large scale from the standpoint of both action and chemical reaction types. They frequently play a role in drug metabolism, as well; in some cases, the product of a biologically active compound is a nitrone, or it is formed as an intermediate step. Nitrones may appear in the biogenesis of endogenous molecules, such as narelin, an alkaloid present in *Alstonia scholaris* leaves [7].

Moreover, these compounds have been shown to affect cellular oxidation state and oxidatively sensitive enzyme systems, but the precise mode of nitrone action has not been elucidated [8–11]. Recent discoveries regarding the activity of nitrones to suppress gene transformation events associated with pathophysiological states, particularly the elaboration of NF kappa B-regulated cytokines and inducible nitric oxide synthesis, argue that nitrones may act at proximal level to oxidatively sensitive signal amplification systems [12, 13].

Radical-induced oxidative damage is extremely harmful to tissues and organs due to molecular modifications caused to polyunsaturated membrane lipids, proteins, and nucleic acids. Oxidative stress is believed to be one of the pathophysiological mechanisms that operate in neurodegenerative disorders such as cerebral ischemias, amyotrophic lateral sclerosis, and Parkinson's and Alzheimer's diseases. Nitrones oppose oxidative challenges by virtue of their ability to very rapidly trap oxygen or carbon centered radicals; thus, generating nitroxide radical species that are more stable and biochemically less harmful than the original radicals. The chemical and pharmacological properties of nitrones depend strongly on the connectivity as well as the type and position of the substituents in the compound's architecture.

The spin adduct formed by nitrogen radical trapping may undergo further transformation inducing reactions with a second radical reduction to hydroxylamines or decomposition to an aldehyde. The generation of nitric oxide in this way suggests that nitrones may be viewed as a means to "package" NO in forms that are better suited to its biological role. Nitric oxide is a bioactive molecule that exerts a number of diverse activities in phylogenetically distant species, as well as opposing effects in related biological systems [14–18]. It was first described in mammals as a major messenger in the

Scheme 1: The most important mechanisms of nitrone synthesis [2]



cardiovascular, immune, and nervous system, in which it plays regulatory, signaling, cytoprotective, and cytotoxic effects. This versatility is mainly achieved through interactions with targets via either a redox or an additive chemistry. In phytobiology, research is only beginning, but recent progresses on NO-functionality in plants are impressive. Obviously, the existence of various types of NO pools, like nitrones, with relative abundance determined, for example, by the pH and redox potential of the microenvironment, provide means through which the transport, lifetime, and targeting properties of the various redox forms of NO can be produced to evoke specific biological responses.

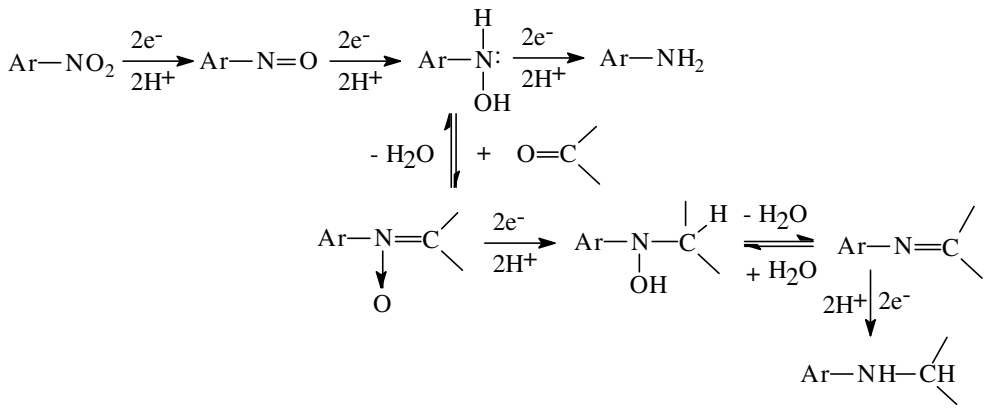
The most important principles of nitrone synthesis are recapitulated in Scheme 1 [2]. One of these methods to obtain nitrones is based on reaction of carbonyl compounds with N-substituted hydroxylamines. This is the easiest and most efficient procedure if the corresponding aldehyde or ketone is not too bulky. The hydroxylamine function can be generated "in situ" from nitro derivatives through reduction by zinc.

2. Investigations, results and discussion

2.1. Chemical-electrochemical synthesis of nitrones

We have elaborated an operative and elegant way for obtaining nitrones, namely the electrochemical reduction of nitro derivatives by polarographic method. Accordingly, in the presence of carbonyl compounds, nitrone is formed by the mechanism presented in Scheme 2 [19]. These reactions take place in successive chemical and electrochemical steps, alternating in the same reaction space: phenylhydroxylamine is formed electrochemically, followed by chemical reactions between this compound and the carbonyl one. Then, the formed oxyazomethine will be reduced by other electrochemical processes and/or will participate in different chemical transformations. Within this context, it is interesting to note that, according to retrometabolic drug design principles [20, 21], nitrones may be considered as a particular case of soft drugs or chemicals owing to their free radical trapping capacity and hydrolysis.

Scheme 2: Mechanism of nitrone formation in the presence of carbonyl compounds



For complete research, we have studied the chemical and electrochemical behavior of the nitrone synthesized in both mentioned conditions. For the nitrone obtained chemically (from phenylhydroxylamine and benzaldehyde), two polarographic waves appear at $E_{1/2} = -0.840$ V and $E_{1/2} = -1.340$ V, respectively. The first belongs to the nitrone itself, and the second belongs to the phenylhydroxylamine and benzaldehyde resulted from the hydrolysis of the principal product (Fig. 1). Phenylhydroxylamine and benzaldehyde are reduced at potentials of similar value. For the nitrone synthesized directly in the polarographic cell, three waves were observed at $E_{1/2} = -0.530$ V, $E_{1/2} = -0.845$ V, and $E_{1/2} = -1.350$ V, respectively. They correspond to the reduction of the nitro group (a fourth electronic wave), to the reduction of the nitrone, and the last one belongs to phenylhydroxylamine and benzaldehyde, overlapped.

To study the kinetics of the complex chemical and electrochemical reactions, it is necessary to know, at least at a comparative level, the rate constants of the component steps, for identification of the rate-determining elementary act of the overall process. In the case of irreversible electrode processes studied by the polarographic method, these parameters may be calculated from data of the adequate i-E curves, or – accepting certain approximations – even from half-wave potentials. Consequently, these last parameters are useful for establishing the order of succession of the electrochemical reactions. As expected, their values are influenced by the substituents introduced in the basic structure (Tables 1 and 2). For component chemical reactions, the rate constants are calculated in the usual way.

The experimental data validate our initial hypothesis of electrosynthesis of nitrones, since the half-wave potentials of chemically and electrochemically synthesized compound are the same. Small differences observed come from the action of the electrode field; eventually, they can appear as a result of the "memory effect" (orientation of molecules derived from a previous electrode process).

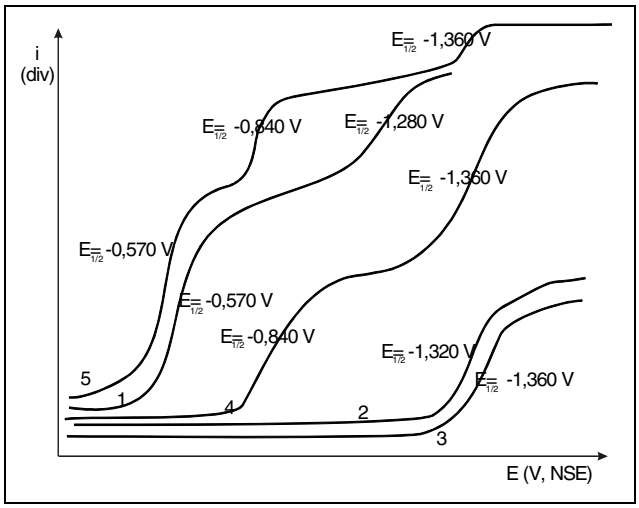
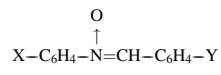


Fig. 1: Polarographic waves of the compounds participating in the formation of nitrones and of the chemical and electrochemical reaction products formed, respectively. Notation: (1) nitrobenzene; (2) phenylhydroxylamine; (3) benzaldehyde; (4) chemically obtained nitrone; (5) electrochemically synthesized nitrone

The generally positive sign of the reaction constants ϱ indicate that the rate determining step is a nucleophilic attack and the nucleophilic agent is an electron. In certain cases when ϱ has negative sign, probably a change takes place in the mechanism, and, therefore, the rate determining step is not the electron-transfer, but, for example, a protonation-transfer. Reactions of this kind are described in the literature. Comparing these constants for both series, it is visible that the susceptibility of the reaction center is in general greater when the substituents are introduced in the benzene ring attached to the azomethinic nitrogen. Consequently, the substituent-effects propagate stronger from this side, since the susceptibility is about twice larger than from the opposite direction (e.g., H: 1.3; *m*-OH: 2.4; *n*-NH_2: 1.6; *p*-NH_2: 2.1; *p*-Cl: 3.0; etc.).

Table 1: Parameters of the regression lines of nitrones

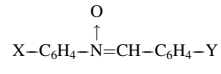


$X-C_6H_4-N=CH-C_6H_4-Y$

Y = H, NH_2, OH, Cl, $N(CH_3)_2$, $NHCOCH_3$

X Param.	H	*m*-OH	*p*-OH	*m*-NH_2	*p*-NH_2	*p*-Cl	*p*-H_3C-CO	*m*-CHO	*p*-CHO	*m*-COOH	*p*-COOH
ϱ (V)	0.098	−0.068	0.090	0.094	0.118	−0.135	−0.046	0.059	0.154	−0.534	0.151
$(E_{1/2})_H$ (V, NSE)	−0.925	−0.892	−0.955	−0.946	0.970	−0.888	−0.856	−0.918	−0.930	−0.817	−0.811
r	0.91	0.93	0.94	0.91	0.95	−0.91	−0.94	0.980	0.985	−0.95	0.91
S_0 (V)	±0.012	±0.011	±0.012	0.017	±0.02	±0.021	±0.005	±0.01	±0.010	±0.028	±0.027

Table 2: Parameters of the regression lines of nitrones



$X-C_6H_4-N=CH-C_6H_4-Y$

X = H, OH, NH_2, Cl, CH_3, COOH

Y Param.	H	*m*-OH	*p*-OH	*m*-NH_2	*p*-NH_2	*p*-Cl	*m*-Cl	*m*-CH_3	*p*-Cl	*p*-$N(CH_3)_2$	*m*-$NHCOCH_3$
ϱ (V)	0.127	0.165	0.192	0.145	0.098	0.401	0.0557	0.017	−0.067	0.058	0.192
$(E_{1/2})_H$ (V, NSE)	−0.854	−0.878	−0.928	−0.906	−1.923	−0.957	−0.954	−0.809	−0.920	−0.972	−1.035
r	0.95	0.92	0.92	0.90	0.91	0.991	0.93	0.962	−0.95	0.90	0.90
S_0 (V)	±0.015	±0.020	±0.035	±0.107	±0.002	±0.020	±0.030	±0.002	±0.072	±0.011	±0.034

These results are in agreement with the mechanism proposed in the literature, namely that the electrode process is initiated by an electrophilic attack of a proton on the oxyazomethinic oxygen. In this way, the reaction center is closer to the aromatic ring having substituents, and the susceptibility of the process presumably will be greater than in the opposite case. Contrary to azomethines, both aromatic rings of nitrones are in the same plane.

The compounds such generated are widely used for obtaining charge-transfer complexes, as well. The polarographic data may be significantly correlated with the complexation parameters. The stability constants collected from the literature [21] for the complexes formed between substituted nitrones and tetracyanoethylene are linearly correlated on a semilogarithmic scale with the half-wave potentials of the corresponding nitrones:

$$E_{1/2} = -0.174\ (\pm 0.030)\ \lg K + 3.54\ (\pm 0.12)\ V \qquad (1)$$

$$n = 5; \qquad r = -0.958; \qquad s_0 = \pm 0.020\ V$$

This is a particular form of linear free energy relations (LFER). Besides its practical value, this and similar equations have a theoretical one, too. Compared with azomethines, in oxyazomethines the electron donor is not the nitrogen (or oxygen) atom but the benzene ring on the aldehyde group side (Fig. 2).

Such independent chemical/electrochemical reactions realized directly in an electrolytic (polarographic) cell can be regarded as a procedure that partially satisfies the requirements of combinatorial chemistry [22]. As it is well-known, combinatorial chemistry proposes two ways for obtaining the compounds: solid-phase (solid/liquid interphase) synthesis and solution (liquid) phase synthesis. In our opinion, in well-defined conditions, the electrode surface may act as a solid supporting phase on which (or in the vicinity of which) the reacting particles are adsorbed, activated, adequately orientated, and undergo their first chemical and/or electrochemical transformations. There is a visible analogy between the electron transfer and the chemical reactions having S_N, S_E, or S_R mechanisms. The products formed may participate in further electrochemical reactions or react with properly selected reagents in the solution. All electrochemically active compounds may be qualitatively and quantitatively characterized by the current-potential curves and the optimal synthesis conditions established. The chosen compound will be synthesized on preparative scale under these conditions. Other information, properties, and parameters may be obtained from Hammett, Taft, Zuman, Hansch etc. type correlations, in which one of the variables is the half-wave potential.

2.2. Hydrolysis kinetics of nitrones

The appearance of aldehyde and phenylhydroxylamine waves on the polarograms indicate that, in parallel with the electrode processes, the hydrolysis of the product takes place and establishes a complex equilibrium. The study of nitrone hydrolysis is of special importance. It is considered that at some cytostatic nitrones their activity mechanism contains basically this reaction step. Comparison of the wave-heights provides a first information about the formation-rate of nitrone as compared to the hydrolysis-rate. For a detailed analysis of the chemical aspect mentioned, we have undertaken a systematic study of the hydrolysis reaction. We followed the variation of the polarographic wave-heights of a chemically prepared nitrone and of its reaction products as a function of time at various temperatures between 20 °C and 50 °C. We have found that with rising temperature the limiting current decreases, that is the hydrolysis-rate must grow. The obtained data permit the calculation of the kinetic order of the hydrolysis and of the rate-constant values. Since the water concentration may be taken as constant, the nitrone hydrolysis may be written as follows:

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B + C$$

and the rate-constant k_1 can be calculated by eq. (2):

$$k_1 = \frac{1}{t}\ \frac{x_\infty}{2a - x_\infty}\ \ln\ \frac{1 - x(1/a - 1/x_\infty)}{1 - x/x_\infty}\ . \qquad (2)$$

Here a is the initial concentration of nitrone, x denotes the concentration of hydrolyzed nitrone, and x_∞ denotes the concentration of the hydrolysis products. From the

$$\left[\lg \frac{1 - x(1/a - 1/x_\infty)}{1 - x/x_\infty}, t\right]$$

dependence, we have calculated the rate-constant k_1 at different temperatures. The equilibrium constant of the hydrolysis reaction is determined from the following formula:

$$K_h = \frac{[C_6H_5-NHOH]\,[C_6H_5-CHO]}{[C_6H_5-NO=CH-C_6H_5]} \qquad (3)$$

or, substituting the concentrations:

$$K_h = \frac{(i_{1/2})^2}{i_2} \qquad (4)$$

where $i_{1/2}$ is the limiting current value corresponding to the overlapped waves of phenylhydroxylamine and aldehyde, respectively. The results given in Table 3 show that the hydrolysis degree of nitrone increases with the rising temperature. Since

$$K_h = \frac{k_1}{k_{-1}} \qquad (5)$$

the rate-constant k_1, i.e., that of the nitrone formation, can be calculated.

The formation rate-constant of nitrone is comparatively small, in agreement with data described in the literature referring to chemically synthesized oxyazomethine. At the mercury electrode, however, this rate-constant is larger.

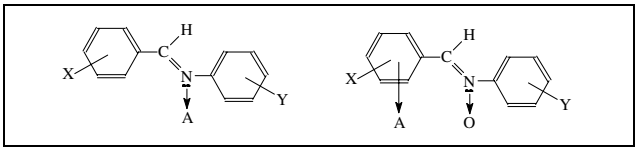


Fig. 2: Compared with azomethines, in the case of oxyazomethines the electron donor is not the nitrogen (or oxygen) atom but the benzene ring on the aldehyde group side

Table 3: Hydrolysis constant, rate constants of the hydrolysis and of the nitrone formation at different temperatures

T (K)	K_h (M)	$k_1 \times 10^4$ (s^{-1})	$k_{-1} \times 10^6$ ($M^{-1}s^{-1}$)
293	40.22	0.0502	0.1250
303	73.25	0.1444	0.1973
313	112.25	0.3193	0.2844
323	125.77	0.8925	0.7096

Under identical conditions, the wave-heights of nitrone obtained both chemically and electrochemically are approximately the same.

The increased rate in polarographic conditions may be explained by supposing that compared to homogeneous medium, some supplementary factors act on the nitrone formation, which facilitates product generation. As a result of the great electric intensity (10^6–10^7 V/m) in the immediate neighborhood of the electrode surface, particles can modify their reactivity by redistribution of electronic density. The molecule is properly oriented at the electrode (Wien-type effect), and its acidic or basic dissociation constant can increase. The orientation in the electrode field generates an induced dipole moment in the molecule, which is added to the permanent one (e.g., in the case of carbonyl group) increasing the molecular polarization and the rate of the reaction compared to that in the solution bulk where this effect is not present. It must be emphasized that the polarization of particles determining the kinetics of processes takes place in the adsorbed state of molecules or in the close vicinity of the electrode. Therefore, phenylhydroxylamine electrochemically formed from both nitrobenzene and benzaldehyde is in activated state and in advantageous position at the electrode surface, and the chemical reaction starts with the attack of basic nitrogen of phenylhydroxylamine on the carbon atom of the carbonyl group. Furthermore, one must take into account that the acid-base equilibrium in the electrode vicinity is displaced toward the acidic form; consequently in the pre-electrode region, pH is 2–3 units smaller than in the bulky one. Electrode processes and the chemical reactions in this space associated with a previous protonation are accelerated when pH decreases. In the case of nitrone formation, protonation is a determining step.

For a more complete image, from the dependence of the hydrolysis equilibrium constant and of the rate-constants on temperature, we have calculated the thermodynamic and the activation parameters of nitrone hydrolysis:

ΔH: 30.60; $\Delta H_1^{\#}$: 71.61; $\Delta H_{-1}^{\#}$: 41.01 kJ/mol

ΔG: −10.30; $\Delta G_1^{\#}$: 24.57; $\Delta G_{-1}^{\#}$: 44.99 kJ/mol

ΔS: 137.3; $\Delta S_1^{\#}$: 157.8; $\Delta S_{-1}^{\#}$: −13.4 J/mol K

The numerical values and their signs agree with the experimentally observed reactivity of nitrones in this reaction.

In conclusion, the synthesis of nitrones is realizable through several combined, successive and/or parallel, chemical and electrochemical steps in the same reaction space. By interpreting the chemical (equilibrium constant, rate constants) and electrochemical parameters (half-wave potentials, limiting currents), one can quantitatively characterize the reactivity of the studied compounds from both thermodynamic and kinetic viewpoints.

The experimental results and their theoretical consequences open new possibilities for a rational optimization of the studied reactions, i.e., increasing the efficiency of some drug and chemical synthesis. The presented chemical/electrochemical reaction-types may be useful from a combinatorial chemistry viewpoint, as well.

3. Experimental

The reference nitrone, phenyl-oxy-azomethine, was synthesized chemically from phenylhydroxylamine and benzaldehyde. The alternate electrochemical-chemical synthesizes, just as the analysis of the products (nitrones) were realized directly in the polarographic cell. The half-wave potentials were determined by comparison with respect to a normal mercury-mercurous sulfate electrode (NSE). Sulfuric acid (0.1 N) in 20% ethanol-water solution was used as background electrolyte. All chemicals used were of analytical grade.

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