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NITROGEN OXIDE GENERATION DURING REDUCTION OF NITROFURAN ANTIBACTERIAL DRUGS

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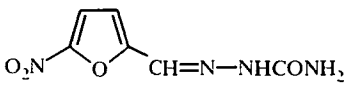
Proof of the nitrogen oxide generation during the chemical reduction of a series of nitrofuran derivatives is obtained by polarographic detection of the nitroprusside anion. A hypothesis is made about the mechanism of the peroxynitrite anion formation, which is responsible for the biological activity of nitrofurans during the course of their reduction.

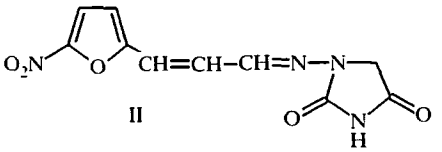
A large group of nitrofurans are used as effective antibacterial preparations [1]. The reductive transformation of the nitro groups by cellular enzymes plays a key role in the biological activity. Several steps in the hypothetical decomposition path of nitrofurans by aerobic microbes include the opening of the furan ring to form acyclic compounds [2].

Reduction of the nitro group would seem to be an important process in the bactericidal activity because the antibacterial activity of the nitrofurans is directly correlated to the energy of the lowest unoccupied orbital and the polarographic reduction potential ($E_{1/2}$) that is closely related to it [3]. Very important information about the properties of these compounds has been reported [4, 5]. It was shown that nitrogen oxide is generated during their reduction. This observation acquires special significance owing to the discovery of endogenic nitrogen oxide that is released by various components of the immune system, including macrophages, from *L*-arginine through the action of NO-synthase and plays an important role as a neurotransmitter [6, 7].

The goal of the present work was to study the formation of nitrogen oxide during the reduction of drugs nitrofural (I), furagin (II), furazolidone (III), and 2-β-ethoxycarbonyl-5-nitrofuran (IV).



I



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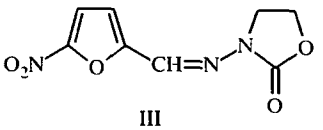

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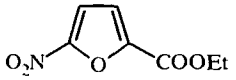

IV

Potassium ferrocyanide and ascorbic acid were used as the reductants. The NO was detected polarographically using the electroreduction peak of the formed nitroprusside ion in the differential-pulse polarograms. We have previously described this method [8].

The electrochemical reduction of the studied compounds on Hg is characterized by successive reduction of the nitro group near -0.1-(-0.6) V (satd. calomel electrode, SCE) and of the azomethine moiety in the 2-position at -0.8-(-1.0) V (SCE) as a function of solution pH. The reduction mechanism has been examined [9] and will not be discussed in the present work.

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A maximum in the differential-pulse polarograms at E = -0.35 V corresponding to a one-electron electroreduction of the generated nitroprusside ion (the height of the maximum increases if sodium nitroprusside is added to the analyzed solution to give a concentration of $2 \cdot 10^{-5}$ M) appears during polarography of 10^{-4} and $2 \cdot 10^{-4}$ M solutions of the studied compounds that are heated to 60°C for 15 min with 10^{-3} M $K_4[Fe(CN)_6]$ at pH 5. A second reduction wave of nitroprusside ion is not observed, probably because the process is inhibited by strong adsorption of the starting compounds or their reduction products. As the concentration of the starting nitrofurans is increased the height of the peak at -0.35 V increases whereas the maxima corresponding directly to the stepwise electroreduction peak of the starting compounds decrease. This is additional confirmation that the maximum is the first electroreduction peak of nitroprusside ion and is indicative of the destruction of the nitro group by the chemical reduction.

The $E_{1/2}$ values for the first reduction wave of the studied compounds in a solution buffered to pH 3.7 [9] and the yields (%) of nitroprusside (average of three experiments) from electroreduction of potassium ferrocyanide (heated at pH 3) are as follows:

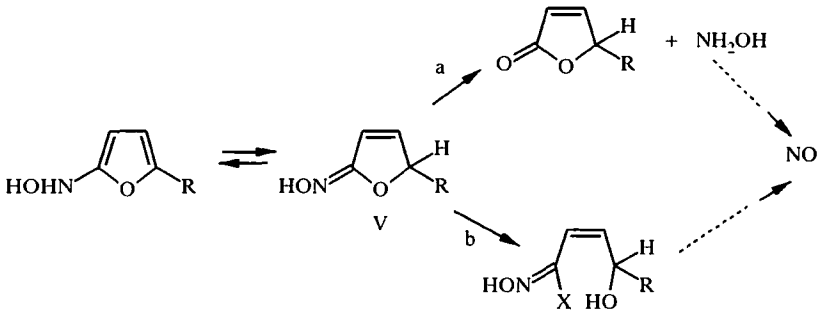
2-β-Ethoxycarbonyl-5-nitrofuran	0.12*	8.5
Furagin	0.07	7.2
Furazolidone	0.12	7.1
Nitrofural	0.10	5.4

* This work.

We observed the formation of the nitroprusside ion in the differential-pulse polarograms also from the chemical reduction of these nitrofurans by ascorbic acid. In this instance the compound was first held for 15 min at pH 6.5 (the optimal condition for reduction in abscorbic acid). Then ferrocyanide was added to bind the resulting nitrite ion.*

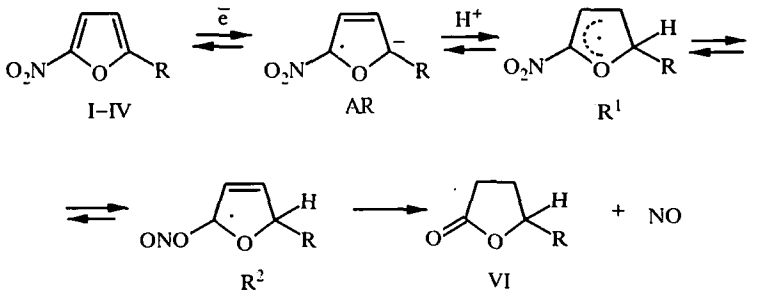
The appearance of an electroreduction wave for nitrite ion at $E_{1/2}$ = -1.2 V in acidic solutions provides indirect proof of the NO formation. The anodic wave of ascorbic acid oxidation at $E_{1/2}$ = +0.02 V decreases simultaneously. These data suggest that NO is generated during the chemical reduction of these antibacterial preparations, in agreement with the literature [4, 5].

A mechanism for the formation of NO during the reduction of nitrofurans has not been published. The NO is probably not released from an oxime intermediate of type V according to paths a and b because our preceding studies [10] have demonstrated that oxidation is required to transform compounds containing oxime into NO.



Literature data indicate that the radicals formed from 5-nitrofurans can undergo a nitro→nitroso ester rearrangement that can eventually lead to NO formation [11]. Based on these data, the steps occuring during the reduction of nitrofurans I-IV can be written as follows:

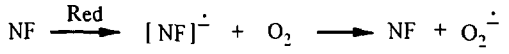
* Nitrite ion is formed as a side product from the reaction of the resulting nitrogen oxide with oxygen from air dissolved in the heated solution according to the reactions: $NO + O_2 \rightarrow NO_2$ and $NO_2 + H_2O \rightarrow HNO_2 + HNO_3$.



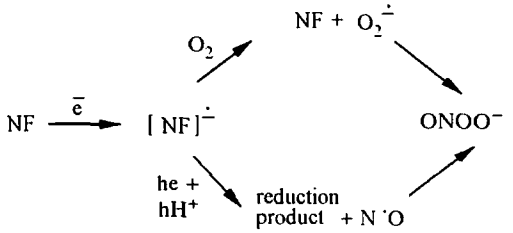
According to this scheme, an anion radical that is protonated to give the radical R^1 is formed during the reduction of compounds I-IV. Then R^1 rearranges into R^2, which transforms into the unsaturated lactone VI and NO. This scheme seems to be correct although the intermediate VI should be isolated and identified in order to prove it.

In conclusion, we would like to express some opinions about the possible mechanism of antibacterial and cytotoxic activity of nitrofurans that is related to the release of NO during reduction. At present it is thought that the peroxynitrite anion ($ONOO^-$), which is produced by various cells of the immune system (macrophages, Kupfer cells, and neutrophils), and not NO is the principal cytotoxic compound. Peroxynitrite is produced by the reaction of two free radicals, NO and superoxide anion. It has been demonstrated [12-14] that the antibacterial activity of peroxynitrite anion is due to inhibition of an electron-transfer chain in mitochondria that is related to the cellular respiration of microorganisms. Peroxynitrite anion also plays a key role in destroying their metabolism.

If the first step in the reduction of nitrofurans (chemical, electrochemical, and biological) is considered to be the formation of an anion radical $[NF]^{\cdot-}$, then superoxide ion can form in an aerobic medium *via* reaction of the anion radical with oxygen:



Superoxide has been detected after reduction of 5-nitrofurans [15, 16]. This suggests that peroxynitrite may form during reduction of 5-nitrofurans according to the scheme:



It seems probable that the formation of peroxynitrite *via* the transformation of nitrofuran into superoxide and NO and their subsequent reaction with each other is a key step that is responsible for the biological activity of nitrofuran.

EXPERIMENTAL

The studied compounds (10^{-4} and $2 \cdot 10^{-4}$ M) were reduced in aqueous citrate–phosphate buffers (pH 3-6.5) and in $HClO_4$ solutions (10^{-3} M) by $K_4[Fe(CN)_6]$ and ascorbic acid (10^{-3} M). The concentration of the nitroprusside ion was determined from the height of its reduction peak in differential-pulse polarograms at E = -0.35 V (SCE). The yield of nitroprusside for a given reduction time (15 min) was calculated using the formula $h_x/h_s \cdot 100\%$, where h_x is the peak height of the formed nitroprusside and h_s is the peak height of the standard addition of sodium nitroprusside. The reduction rate of the nitrofurans was determined from the decrease with time of the anodic oxidation wave of ascorbic acid in constant-current polarograms.

Differential-pulse polarograms were recorded on a PU-1 polarograph (Belarus). Amperometric determinations were made on a ON-105 polarograph (Hungary). Working electrodes were a Hg-drop electrode with a plate (parameters: τ = 0.2 sec, m = 1.2 mg/sec) and a Hg-drop electrode (τ = 3 sec, m = 0.9 mg/sec). The reference electrode was SCE. The polarograms were measured in a thermostatted cell at 20°C. Furacin, furaginum, and furazolidone were pharmaceutically pure. The purity of 2-β-ethoxycarbonyl-5-nitrofuran was 99%. Reagents ($K_4[Fe(CN)_6]$, ascorbic acid, and background electrolytes) were chemically pure grade. Solutions were prepared with distilled water.

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