STABILITY OF RADICAL ANIONS DERIVED FROM SUBSTITUTED 5-NITROFURANS INVESTIGATED BY ESR SPECTROSCOPY

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It has been found that the decay in dimethylformamide and dimethylformamide-water mixtures of radical anions in five of the investigated 5-nitrofurans is governed by a second-order reaction. Only the decay of the radical anion generated from 5-nitro-2-furfural III may be described by an equation including parallel first- and second-order reactions; this behaviour is evidently caused by the relatively high stability of the corresponding dianion, this being an intermediate in the reaction path. The presence of a larger conjugated system in the substituent in position 2 results in a decrease of the unpaired electron density in the nitro group and, consequently, an increase in the stability of the corresponding radical anions.
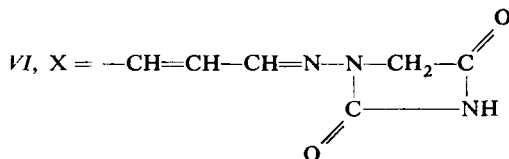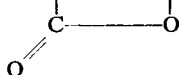
The polarographic[1], cyclic voltammetric[2] and ESR[3] investigation of redox properties of substituted 5-nitrofurans present a proof that the primary reduction products in these compounds are radical anions resulting from the attachment of a single electron to the starting molecule. The products of such a one-electron transfer were also observed by Mason and coworkers[4] in their enzymatic reduction. The stability of the pertinent radical anions is one of the important properties determining the radiosensibilizing activity of 5-nitrofuran derivatives when used in treating cancer[5,6]. It has been shown[7] that the radical anions resulting from simple 5-nitrofuran derivatives are poorly stable in water or in water–ethanol mixtures and their stability strongly depends both on the character of the substituent and on the medium.

The aim of this communication was the stability determination of radical anions of some 5-nitrofuran derivatives in aprotic media (dimethylformamide, DMF) and in DMF–water mixtures. The following substances were investigated: 5-nitrofuran (*I*), 2-methyl-5-nitrofuran (*II*), 5-nitro-2-furfural (*III*) and the pharmaceuticals Nitrofurazon (N-(5-nitro-2-furylmethyleneamino)urea, (*IV*)), Furazolidon (3-(5-nitro-2-furylmethyleneamino)-1,3-oxazolid-2-one (*V*)), and Furagin (1-(3-(5-nitro-2-furyl)-alkylidene amino)-1,3-diazol-2,4-dione (*VI*))*.

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* The potassium salt of Furagin (Solafur, soluble Furagin) was used which is better soluble. It follows from its dissociation constants ($pK_a = 12.9$ in DMF and $pK_a = 6.0$ in water) that the substance is dissociated in the solution.

$$O_2N \quad O \quad X$$
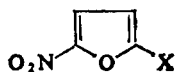
I, X = H
II, X = CH_3
III, X = CHO
IV, X = —CH=N—NH—CONH_2
V, X = —CH=N—N—CH_2—CH_2
$$\quad\quad\quad\quad\quad\quad C\text{————}O$$
$$\quad\quad\quad\quad\quad O$$

VI, X = —CH=CH—CH=N—N—CH_2—C
$$\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad\quad C\text{————}NH$$
$$\quad\quad\quad\quad\quad\quad\quad O$$

EXPERIMENTAL

Substances *I— VI* were synthesized by procedures described in the literature[8–12]. Dimethylformamide was purified by azeotropic distillation with benzene and water at atmospheric pressure. Before use it was rectified at reduced pressure. The water concentration (measured by chromatography) was lower than 10^{-2} mol l^{-1}. n-Tetrabutylammonium hexafluorophosphate (0·1 mol l^{-1}) was used as supporting electrolyte. The concentration of the substances under study was usually 1 to 2 . 10^{-3} mol l^{-1}.

The electrochemical measurements were performed with the polarograph GWP 673 (produced in the German Democratic Republic). The ESR spectra were recorded with the Varian E-4 spectrometer at constant temperature.

Measuring Procedures

The stability of products in electrochemical reactions is often investigated by cyclic voltammetry with a hanging mercury drop electrode (HMDE) (ref.[13,14]). In our case this method did not lead to satisfactory results due to a too high stability of the studied radicals; at low polarization rates the solution convection around the electrode became pronounced, at higher rates too low concentration changes occurred during one cycle. In some cases also the influence of the heterogeneous reaction became evident. This is why ESR spectroscopy with electrochemical radical generation outside the cavity followed by a transport of the solution to the cavity was applied. The advantage of this arrangement in comparison with the formerly used simpler arrangement comprising a generation directly in the cavity[15,16] is based particularly on the homogeneous distribution of radical concentration in the cavity. The homogeneity of the solution is necessary in our case since the radical decay is not controlled by a first order process.

The main part of the assembly for external radical generation is shown in Fig. 1. The electrolytic cell *1* forms a single unit with the reservoir *2*; both parts are connected by a teflon stopcock *3*. The working electrode is formed by mercury *4* filling up the bottom part of the electrolysis cell. The area of the working electrode is about 1·5 cm^2. The reference electrode is connected with the

solution in the inlet into the cell by a solvent bridge with porous carbon 5. The inlet is terminated by a capillary above the working electrode. The auxiliary electrode 6 is placed in the detachable part 7. It is connected with the solution *via* a glass frit. This electrode is formed either by a carbon rod or a platinum mesh. The outlet 8 from the cell is just below the level of the frit separating the space containing the auxiliary electrode. The solution is led from the electrolytic cell into a ESR flat quartz cell (Varian V-4548) placed in the resonator of the ESR spectrometer (Varian E94) by a teflon tube with 0·5 mm diameter and about 150 mm length. With respect to the fact that oxygen may diffuse through teflon the tubing was covered with another tubing made of tygon which was continuously flushed with an inert gas. From the ESR cell the solution may flow through the teflon tube to the reservoir.

The volume of the solution at the working electrode is given by the mercury level height. Usually, we were working with approx. 1·5 ml. The duration of the electrolysis was between 60 and 600 s. The "dead volume", *i.e.* the volume of the connection from the space of the working electrode to the cuvette increased by the volume of the bottom part of the cuvette is about 0·15 ml.

During electrolysis the solution was stirred by moving the whole electrolytic cell. The current ranged from 50 to 200 mA depending on the stirring efficiency. On terminating the generation the solution was transported to the measuring cuvette by raising the working electrode. In this situation, the whole volume of the electrolysis cell and that of the connection with the flat cell was filled up by mercury. In this way the back-flow of the solution through the frit separating the compartment with the auxiliary electrode was made impossible. When measuring concentration c_x (or its time-dependence, *i.e.* in kinetic plots) by means of ESR, the following relationship is used:

$$c_x = \frac{kA}{RG \cdot MA \cdot \sqrt{P}} A \,, \tag{1}$$

where k is a constant which may be determined with the help of a standard possessing a known concentration, RG is the receiver gain of the instrument, MA the modulation amplitude, P the applied microwave power, and A is the area underneath the absorption curve.
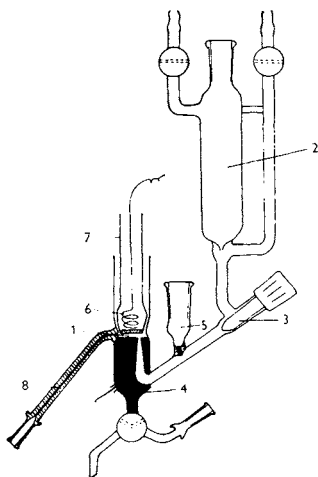

FIG. 1

Electrolytic part of the assembly for external electrochemical radical generation at a mercury electrode: **1** electrolytic cell; **2** reservoir; **3** teflon stopcock; **4** mercury working electrode; **5** solution bridge for attaching the reference electrode; **6** auxilliary electrode; **7** detachable part with the auxiliary electrode; **8** outlet from the electrolysis cell

Because of the fact that ESR spectra are recorded in the form of their first derivative, a double integration of the spectra is necessary for determining the area A. In case of radical anions of compounds $I-V$ this task was facilitated by the fact that a separate line could be found in their spectra whose intensity (the area A_k) was a known part of the whole spectra intensity, *i.e.*

$$A_k = \frac{A}{R_k}, \tag{2}$$

where the constant R_k may be determined on the basis of spectrum analysis. Since the shape of the curve (Lorentzian) is known, it suffices for determining the area A to know also the width of the line ΔH_{pp} and its amplitude I'_{pp}

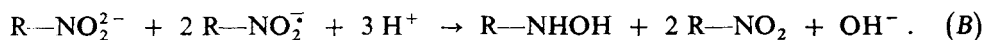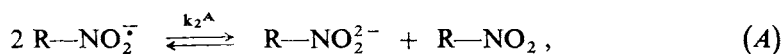$$A = R_k A_k = R_k \frac{\pi}{\sqrt{3}} (I'_{pp})_k (\Delta H_{pp})_k^2. \tag{3}$$

When studying the decay kinetics of radical anions of compounds $I-V$ such an independent line was repeatedly recorded. A more complicated situation developed with radical anions of Furagin (VI). Its spectrum does not contain any sufficiently intensive independent line so that for investigating kinetics it would be necessary to perform repeatedly a double integration of the whole spectrum. This was impossible in our case because the registration of such a spectrum with a good resolution requires too much time. This is why we reached a broadening of the lines by a partial saturation of the transitions by increasing the microwave power. In this way the influence in the change of line widths as a function of concentration change was decreased. Moreover, broadening of the line width leads to a simplification of the spectra whose shape remains unchanged both by the changes in radical anion concentrations and by changes in water concentrations in the solution to be investigated. The decay kinetics of the radical anions of VI were then studied from the changes in the amplitude of this spectra with time. In this way one may obtain relatively good data about the shape of the concentration — time plot and about the relative values of the rate constants of radical anion decay in different media (at different water concentration). However, the absolute value of the rate constant is encumbered with a large error. Since even at a partial saturation of the transitions the value of the signal does not further grow as a linear function of the square root of the microwave power but slower, the relationship (I) is no more exactly valid. The actual concentration of radical anions of VI is thus higher (and, consequently, the second order rate constant lower) than the values obtained from (I).

RESULTS AND DISCUSSION

It follows from the linear plot of the reciprocal value of concentration on time that the radical anions decay of I, II, IV, V and VI may be described by a second-order relation. The radical-anion of III is controlled simultaneously by reactions of the first and of the second order. The obtained effective rate constants k_2^{ef} or k_1^{ef} of the model substances $I-III$ are shown in Table I, the rate constants for those applied as pharmaceuticals $(IV-VI)$ may be found in Table II.

The studied radical anion decay may be described on the basis of a mechanism proposed by Kastening[17] for the nitrobenzene radical anion decay. Kastening's scheme contains two parallel paths:

A) radical anions dismutation with a follow-up reaction of the resulting dianion:

$$2 \; R\text{—}NO_2^{\cdot-} \; \underset{}{\overset{k_2^A}{\rightleftharpoons}} \; R\text{—}NO_2^{2-} + R\text{—}NO_2 , \qquad (A)$$

$$R\text{—}NO_2^{2-} + 2 \; R\text{—}NO_2^{\cdot-} + 3 \; H^+ \rightarrow R\text{—}NHOH + 2 \; R\text{—}NO_2 + OH^- . \qquad (B)$$

B) protonation of the radical anion, an electron uptake from the unprotonated (adical anion and a follow-up reaction analogous to (B):

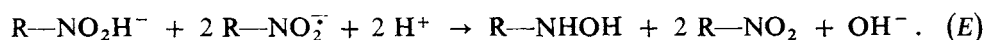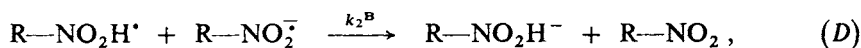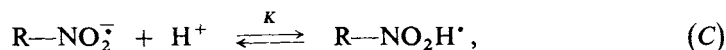$$R\text{—}NO_2^{\cdot-} + H^+ \; \underset{}{\overset{K}{\rightleftharpoons}} \; R\text{—}NO_2H^\cdot , \qquad (C)$$

$$R\text{—}NO_2H^\cdot + R\text{—}NO_2^{\cdot-} \; \overset{k_2^B}{\longrightarrow} \; R\text{—}NO_2H^- + R\text{—}NO_2 , \qquad (D)$$

$$R\text{—}NO_2H^- + 2 \; R\text{—}NO_2^{\cdot-} + 2 \; H^+ \rightarrow R\text{—}NHOH + 2 \; R\text{—}NO_2 + OH^- . \qquad (E)$$

TABLE I

The effective rate constants k_2^{ef} (l mol^{-1} s^{-1}) and k_1^{ef} (s^{-1}) of the model substances *I—III*

c_{H_2O} mol l^{-1}	*I* (X = H) k_2^{ef}	*II* (X = CH$_3$) k_2^{ef}	*III* (X = CHO) k_2^{ef}	$k_1^{ef} . 10^4$
0·01	11	8·1	3	0·71
1·0	16·5	10·5	13·5	0·67
2·5	—	—	29	0·78
5·0	37	24	250	3·6
10·0	170	28	7 700	3·2
20·0	1 840	87	—	—

TABLE II

The effective rate constants k_2^{ef} (l mol^{-1} s^{-1}) of the pharmaceuticals *IV— VI*

c_{H_2O} mol l^{-1}	*IV* (Nitrofurazon)	*V* (Furazolidon)	*VI* (Furagin)
0·01	2·0	0·3	—
2·0	11	3·5	0·03
5·0	33	17	—
8·0	—	41	1·1
10·0	120	87	3·9
20·0	460	350	17

Both reaction paths lead to the same products according to the overall chemical equation

$$4\ R\text{—}NO_2^{\bullet-} + 3\ H^+ \quad \rightarrow \quad R\text{—}NHOH + 3\ R\text{—}NO_2 + OH^- \ . \qquad (F)$$

Assuming that the protonation rates (C) and those of the follow-up reactions (B) and (E) are high in comparison with the dismutations (A) and (D) the following relationship results for the effective rate constant of a second order reaction

$$k_2^{ef} = 4k_2^A + 4k_2^B\ K[H^+] \ . \qquad (4)$$

In all studied 5-nitrofuran radical anions, the effective rate constant k_2^{ef} increased with increasing water concentration in a non-linear way $(Fig.\ 2)$. On the one hand this is caused by the fact that the hydrogen ion concentration is not directly proportional to the water concentration because the water dissociation degree is a function of its concentration; moreover, the protonation equilibrium (C) characterized by the constant K includes not only the protonation by dissociated hydrogen ions but also the possible direct reaction of radical anions with water. It is also probable that the rate constants k_2^A and k_2^B of dismutations (A) and (D) vary with a change in solvation.

Substance *III* reduces even in dimethylformamide with a small addition of water in two reversible one-electron steps[18]. The carbonyl group in position 2 causes
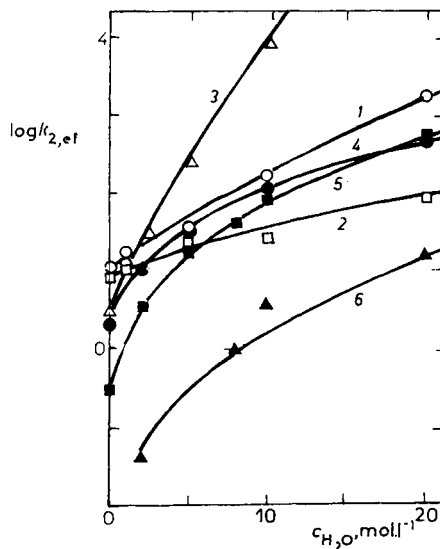

FIG. 2

Dependence of the effective rate constant k_2^{ef} (logarithmic scale) in the decay of radical anions of substituted 5-nitrofurans on water concentration in dimethylformamide. *1* 2-Nitrofuran (*I*); *2* 2-methyl-5-nitrofuran (*II*); *3* 5-nitro-2-furfurol (*III*); *4* Nitrofurazon (*IV*); *5* Furazolidon (*V*); *6* Furagin (*VI*)

namely an increased stability of the dianion by enabling the formation of a quinoid structure. Regarding the relative stability of the dianion of this compound the assumption concerning the high rate of its decay (reaction (B)) in comparison with dismutation (A) is not valid (this assumption holds with the radical anions of the other investigated 5-nitrofurans in which either the formation of dianions is not observed or appears only in anhydrous dimethylformamide). Consequently, the rate-determining step in III is not only the dismutation (A) but also the dianion decay (B). This is why the decay of the radical anion of III has another course than the radical anion decay in the other compounds of this series. This manifests itself both in the appearance of a reaction of another (first) order, and in a different (steeper) dependence of the rate constant k_2^{ef} on water concentration.

On comparing the values of rate constants k_2^{ef} of compounds IV and VI and of the model substances I and II one sees that the electron distribution over the larger conjugated system of the substituent in position 2 leads to a decrease in the value of the rate constant k_2^{ef}, i.e. to an increase in the stability of the corresponding radical anions. The distribution of the unpaired electron also affects the values of hyperfine structure splitting constants. On comparing the splitting constants a_N of the nitrogen nucleus in the nitro group of substances $IV-VI$ $(a_N^{VI} = 0.6$ mT, $a_N^V = 0.7$ mT, $a_N^{IV} = 0.74$ mT) (ref.[19]) with the splitting constant a_N of 2-nitrofuran I $(a_N^I = 0.99$ mT) (ref.[20]) the same sequence of magnitudes results as in rate constants:

$$a_N^{VI} < a_N^V < a_N^{IV} < a_N^I$$
$$(k_2^{ef})^{VI} < (k_2^{ef})^V < (k_2^{ef})^{IV} < (k_2^{ef})^I .$$

It is evident that substitution in position 2 by a substituent with a large conjugated system may yield 5-nitrofuran with considerably stable radical anions.

On the contrary, the introduction of a methyl group, i.e. of an electron donating group into position 2, leads to an increased value of a_N. However, the decrease in the stability of the radical anion of II in comparison with I does not occur. It is assumed that this is due to a larger rigidity of the π-electron system of the radical anion of II (ref.[20]); this situation makes more difficult the uptake of a second electron to the radical anion from another radical anion in the dismutation reaction:
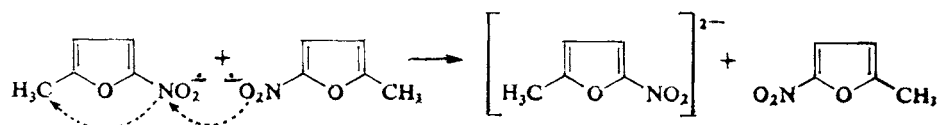
The greater rigidity of the π-system in 2-methyl-5-nitrofuran (II) follows also from the lower sensitivity of hyperfine structure constants toward a change in the solvent

(when passing from dimethylformamide to water a_N^I changes by $0 \cdot 356$ mT, whereas a_N^{II} only by $0 \cdot 244$ mT).

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