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REACTION OF VINYLFURANS WITH SULFHYDRYL AND AMINO GROUPS

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Derivatives of 5-nitrofuran have been used as antimicrobial agents in clinical and veterinary medicine, as preservatives in human food and as additives to feed for livestock. Recent studies with nitrofurans revealed that some of them are mutagenic in microorganisms and carcinogenic in laboratory mammals [1 - 5]. From the point of view chemotherapy the most interesting derivatives are those of vinyl and azomethine type. Mechanism of vinylfurans (VF) action has not been studied in detail and in the case of the azomethine derivatives there exist only ununified conceptions as well [6 - 9]. A new view on the mechanism of VF action afforded our findings that this compounds are effective inhibitors of bioenergetic processes (glycolysis and mitochondrial functions) due to chemical modification of functional thiol groups of some enzymes [10]. Based on these findings we expressed an opinion that for the elucidation of biochemically important reactions of VF the reactions with nucleophilic groups are of primary importance. Similar reactions we suppose for the furan derivatives of azomethine type (with activated exocyclic $\succ C = N -$ double bond).

The investigation of the reactions with benzyl mercaptan in methanol at room temperature was first step in the study of reactions between VF and thiols. Using the thin layer chromatography (silica gel plates and benzene as developing agent) the formation of new products was observed. These products were prepared as follows : 5 mmoles of apropriate VF were dissolved in 15 ml of dry methanol. After adding 4 mmoles of benzyl mercaptan the reaction mixtures were stirred at laboratory temperature for 24 h. Then the solvent was removed in vacuo and products were recrystalized from dry ether (50 - 80 % yields). Kinetic measurements of the VF reaction with thiols and glycine were carried out spectrophotometrically (wavelenghts = 300 - 500 nm) in 0.1 and 0.2 M Clark-Lubs buffered reaction mixtures (pH = 4 - 10) containing at least a 20 - fold excess of nucleophiles. Under these conditions the reactions are pseudomonomolecular and obeyed the equation of the first - order reactions (time dependence of the values $\log \Delta A$ is linear). VF were dissolved in methanol. The resulting aqueous buffered reaction mixtures contained 1 % of methanol by volume. The first - order rate constants,

k_{obs} $[s^{-1}]$, were obtained from the slopes of the linear dependence of log $(A_t - A_\infty)$ plotted against time after calculating according to equation 1 :

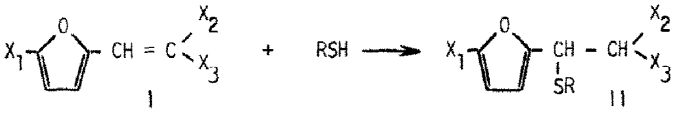
$$k_{obs} = 2.3 \left[\log (A_{t_2} - A_\infty) - \log (A_{t_1} - A_\infty)\right](t_2 - t_1) \qquad (1)$$

The second - order anion rate constants $\underline{k}$ $[M^{-1}s^{-1}]$ were calculated from equation 2 :

$$\underline{k} = k_{obs} (K_a + C_{H^+}) C_o^{-1} K_a^{-1} \qquad (2)$$

where K_a is the dissociation constant of the thiol, C_o $[M]$ its initial analytical concentration, and C_{H^+} $[M]$ is the concentration of the H^+ ions in buffered systems. The further details see in our previous paper [11]. Recording absorption spectra and the kinetic measurements were carried out with Specord UV - VIS spectrophotometer, Zeiss, Jena and SP 30 reaction rate system Unicam, Cambridge.

The reaction of vinylfurans (VF; compounds in Tab. 1 of structure I) with thiols in aqueous buffered systems gives addition products of general structure II according to the following schematic equation [Eq. 3]:



(3)

In the presence of excess of thiol, the reactions are of first order with respect to concentration of VF and may be spectrophotometrically investigated. In the presence of a constant initial concentration of thiol the reaction rate (i.e., first order rate constants, k_{obs} $[s^{-1}]$) increases with pH value of the reaction mixture and is governed by concentration of the thiolate anion. Owing to this fact, the corresponding second order anion rate constants $\underline{k}$ $[M^{-1}s^{-1}]$ have practically equal values (Fig. 1). The data in Tab. 1 enable us to compare the reactivity of the investigated VF to thiols. For derivatives No. 2,3,4, the values of $\underline{k}$ $[M^{-1}s^{-1}]$ indicate a positive influence on reactivity of the introduction of electron withdrawing substituents into position 5 of the furan skeleton. Similarly, a positive effect may be achieved by analogous substitution on the exocyclic double bond (see derivatives No. 4,5). As for the reactivity of thiols to VF, it is governed by their basicity (Tab. 2).

The above results show that the reactions between the VF investigated and thiols are nucleophilic addition reactions (Ad_N). This conclusion is also supported by elemental and NMR analysis of the reaction products of two VF derivatives, No. 1 and 6, with benzyl mercaptan. The ^{1}H-NMR spectrum in $CDCl_3$ of the product obtained from the substance No. 1 exhibited peaks at δ 3.50 - 3.76 (2H,m), δ 4.25 - 4.76 (3H,m), δ 6.05 - 6.28 (2H,m) and δ 7.09 - 7.46 (6H,m). The content of C,H,N,O and S in this product found by elemental analysis and calculated for $C_{13}H_{13}N_1O_3S_1$ was as follows. Found : C 59.40; H 4.89; N 5.27; O 18.22; S 12.22. Calculated : C 59.36; H 4.93; N 5.32; O 18.24; S 12.15. The ^{1}H-NMR spectrum in $CDCl_3$ of the product obtained from the substance No. 6 exhibited peaks at δ 1.00 - 1.51 (6H,m), δ 3.75 - 4.63 (8H,m), δ 6.20 - 6.38 (1H,d) and δ 7.10 -

-7.51 (6H,m). The content of C,H,N,O and S in this product found by elemental analysis and calculated for $C_{19}H_{21}N_1O_7S_1$ was as follows. Found : C 56.10; H 5.12; N 3.49; O 27.45; S 7.81. Calculated : C 56.01; H 5.19; N 3.44; O 27.49; S 7.87.

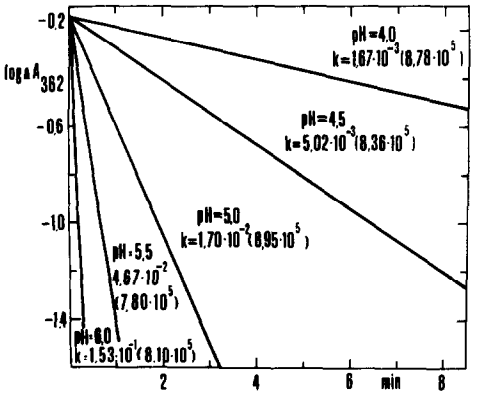


Fig.1. Course of the reactions of the compound No.5 with 2-mercaptoethanol in media with different pH values (0.1 M Clark-Lubs phthalate buffers) as investigated spectrophotometrically at 362 nm. Numbers over the lines are the calculated values of the first order rate constants, k_{obs} $[s^{-1}]$ while the numbers in parentheses are the second order anion rate constants $\underline{k}$ $[M^{-1}s^{-1}]$. A = absorbance

Table 1. Second order rate constants for the reactions of vinylfurans with thiols and glycine. The substituents are related to general structure I [Eq. 3].

Compound No.	Substituents X_1	X_2	X_3	k $[M^{-1}s^{-1}]$ at 25°C [a] Thiol[b]	Glycine[c]
1	H	NO_2	H	2.3×10^5	1.8×10^0
2	H	NO_2	$COOCH_3$	9.2×10^4	3.0×10^1
3	Br	NO_2	$COOCH_3$	2.7×10^5	5.2×10^1
4	NO_2	NO_2	$COOCH_3$	4.5×10^6	3.9×10^2
5	NO_2	NO_2	H	8.5×10^5	8.2×10^0
6	NO_2	$COOC_2H_5$	$COOC_2H_5$	6.7×10^3	1.2×10^0
7	NO_2	$COOC_2H_5$	H	5.4×10^2	-
8	NO_2	2-furyl	$CONH_2$	1.2×10^0	-

[a] The rate constants represent average values of three measurements, the deviations from which did not exceed 5 %. The dissociation constants, pK_a, for 2-mercaptoacetic acid (10.11), 2-mercaptoethanol (9.48) and glycine (9.78) were taken from [11, 12].

[b] Reactivity of compounds No. 1,7,8 was characterized to thioglycolic acid in 0.1 M borate Clark-Lubs buffer pH 10.0. Reactivity of other derivatives was checked with 2-mercaptoethanol in 0.1 M phosphate Clark-Lubs buffer, pH 6.0 (that of compound No.4 in 0.1 M phthalate Clark-Lubs buffer pH 4.0). Initial concentration of vinylfurans was 5×10^{-5} M and three variable concentrations of thiols in the range 5×10^{-4} - 5×10^{-3} M were used.

[c] Reaction in 0.2 M Clark-Lubs borate buffer, pH 9.3. Initial concentration of VF was 5×10^{-5} and that of glycine was 5×10^{-3} - 1×10^{-2} M.

Attention was focussed on these two products with regard to their isolation and structure determination. On the basis of our preceeding results, we compared the reactivity of VF to thiols with that of some known thiol-combining agents. The values of the second order anion rate constants k $[M^{-1}s^{-1}]$ for the reactions of these substances with cysteine at 25°C are [15]: iodoacetamide - 1.0, acrylonitrile - 1.7, benzyl isothiocyanate - 27.9, phenyl isothiocyanate - 586, N-ethylmaleimide - 3050, 5,5'-dithio-bis(2-nitrobenzoic acid) - 41 000. The reactivity of the derivative No. 5 is 70 600 (Tab. 2) as determined in an analogous way.

Table 2. Reactivity of 1-(5-nitro-2-furyl)-2-nitroethylene [compound No. 5] to thiols in 0.1 M Clark-Lubs buffer, pH 4.4, at 25°C.

Thiol	pK_a [ref.]	k $[M^{-1}s^{-1}]$
Cysteine	8.10 [13]	7.06×10^4
Benzylmercaptan	9.43 [14]	6.58×10^5
2-Mercaptoethanol	9.48 [11]	8.55×10^5
Mercaptoacetic acid	10.11 [11]	9.32×10^6

The inhibitory effect of VF especially on thiol enzymes may be also explained by their reactivity to thiols. Fig. 2 indicates a rapid inactivation of the rabbit muscle D-glyceraldehyde--3-phosphate dehydrogenase (GAPDH; EC 1.2.1.12) by derivative No. 4 at 25°C. The reaction mixture was 0.1 M carbonate-hydrocarbonate buffer of pH 8.6, 0.2 mM EDTA, 1.5 mM GAP, 50 mM $P_{inorg.}$, 1.5 mM NAD, and rabbit muscle GAPDH (1.6 μg protein/ml). Under above conditions, the inhibition constant of the compound No. 4 is K_i = 4.97 μM, as calculated from a Lineweaver-Burk plot of initial velocites for variable concentration of NAD.

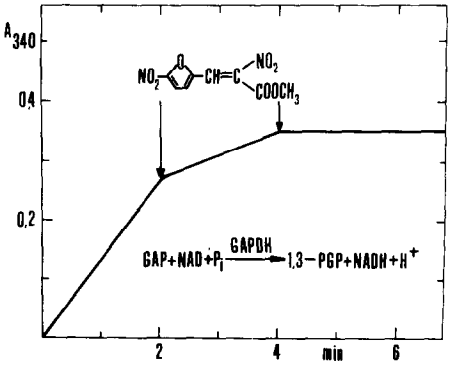


Fig.2. The inactivation of glyceraldehyde--3-phosphate dehydrogenase by compound No.4. The activity was measured by recording the increase in absorbance at 340 nm ($NADH_2$ formation). Arrows indicate the addition of inhibitor necessary to obtain the final concentration of 40 and 80 μM respectively. A = absorbance.

The quantitative data concerning the reactivity of VF to glycine are listed in Table 1. They indicate a lower reactivity of glycine (in its RNH_2 form) in comparison with thiols (in their RS^- forms). The values of the second order rate constants are by 3 - 5 orders of magnitude higher for thiols. These experimental findings are in good agreement with theoretical considerations on the reactivity of different nucleophilic agents [15, 16]. As the nucleophilicity of the amino group of glycine is comparable with the nucleophilicity of the OH^- ion, it may be suggested that VF reacts with OH^- ions in alkaline solutions. In case of the investigated reactions of VF with thiols and glycine, this competetive reaction with the OH^- ions does not occur because of the experimental conditions (pH and excess RS^- or RNH_2 with respect to the OH^- concentration).

The aim of this preliminary communication is to direct attention to the nucleophilic addition reactions of VF with the thiol and amino groups. These reactions have not been hitherto taken into consideration in relation with the biological activity of VF. That also concerns 3-(5-nitro-2-furyl)--2-(2-furyl)- acrylamide (AF_2, compound No. 8) which is "well known" as a biologically active species. We suppose that the antimicrobial and almost cytotoxic effect of VF might be at least partly explained by modification of the proteins thiol groups. On the other hand, the suggested reactivity to other nucleophilic groups could be a clue to the elucidation of more specific effects of VF, e.g. their mutagenity.

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