REACTIVITY OF 2-FURYLETHYLENES WITH NUCLEOPHILIC GROUPS AND ITS BIOLOGICAL SIGNIFICANCE

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Received February 14th, 1986

The paper describes addition-nucleophilic reactions of 1-(5-R^1-2-furyl)-2-R^2-R^3-ethylenes with SH (thioglycolic acid), NH_2 (glycine) and OH (phenol) nucleophiles, and with hydroxyl ions in aqueous media. The determined rate constants, of second order ($dm^3\ mol^{-1}\ s^{-1}$), show that in the physiological conditions the preferential reaction will be that with thiols. The amino acid composition of proteins modified with 1-(5-nitro-2-furyl)-2-nitro-2-methoxycarbonylethylene has revealed that the only group affected was the SH group of cysteine. This finding points out the significance of addition-nucleophilic SH-interactions of 2-furylethylenes with the protein macromolecules and accounts for the high cytotoxicity of these compounds.

Derivatives of 2-furylethylene have been known for decades as biologically active substances. The strong mutagenic and carcinogenic activity of 5-nitro-2-furylethylenes[1-4] is due to reduction of the nitro group in the 5-position of the furan ring, giving rise to intermediates highly reactive with nucleic acids[5]. Substituents in the 2-position of the furan ring influence only the extent of this biological response[6] (by altering the lipohydrophilic properties of the molecule). By contrast, the demonstrated antimicrobial and cytotoxic effects of 2-furylethylenes[7-9] consist in their capability of addition-nucleophilic reactions. In these reactions 2-furylethylenes modify the nucleophilic groups of low-molecular-mass compounds, but also of enzymes, which leads to loss of their catalytic activity and to elimination of processes affording energy to cells. The objective of the present study was to find out which functional groups react preferentially with furylethylenes. We studied reactions of selected derivatives of 2-furylethylene with low-molecular-mass SH, NH_2, and OH^- nucleophiles, as well as interactions of the 2-furylethylenes with nucleophilic groups of model proteins.

EXPERIMENTAL

Chemicals and biopolymers. All the 2-furylethylenes studied (Table I) were synthesized at the Department of Organic Chemistry, Slovak Institute of Technology, Bratislava. Compounds *I*—*VI* and *IX* were obtained by a described procedure[10]. Compound *VIII* was obtained by condensation of furfuraldehyde with nitromethane[11]; nitration of *VIII* according to Sazaki[12]

afforded *VII*. Yeast alcohol dehydrogenase was a Sigma product (St. Louis, U.S.A.), bovine serum albumin a Merck product (Darmstadt, F.R.G.).

Methods. The UV and VIS spectra of 2-furylethylenes and their reaction products with model —SH, $—NH_2$, and —OH nucleophiles were measured employing a Specord UV-VIS spectrophotometer (Zeiss, Jena) in buffers according to Clark and Lubs[13] (0·2 mol dm^{-3}, pH 5—9·3) and Briton and Robinson[14] (pH 12) at 25°C. The pH's were adjusted using a pH-meter OP 207 (Radelkis, Hungary). The 2-furylethylenes were added from stock solutions in dimethyl sulphoxide or dioxan to a final concentration of the solvent in the mixture 0·5 vol. %. The initial concentration of the 2-furylethylene for kinetic measurements was $5 . 10^{-5}$ mol dm^{-3}; the model thiol, amine, alcohols and OH^- ions were used in a concentration range of $5 . 10^{-3}$ to $1 . 10^{-1}$ mol . . dm^{-3}. Under these conditions (>20 fold excess of a nucleophile over a 2-furylethylene) the reactions proceeded as pseudomonomolecular ones. The kinetics were measured in the region of absorption peaks of the derivatives studied (340—370 nm), where the other reactants (thiols, amines, alcohols) did not absorb. The second-order rate constant was calculated from the equation[15]

$$k = k_{obs}(K_a + c_{H+})/c_0 K_a ,$$

where K_a designates the dissociation constant of the nucleophile, c_0 (mol dm^{-3}) the initial analytical concentration of the nucleophile, c_{H+} (mol dm^{-3}) the concentration of protons and k_{obs} (a first-order rate constant) the slope of the linear plot of $\ln (A_t - A_{t+\Delta t})$ *vs* t (A_t denotes absorbance at time t, Δt a suitable time interval).

Amino acid analysis: 10 mg of the alcohol dehydrogenase or bovine serum ablumin was dissolved in 5 ml of distilled water and the derivative *I* was added ($1 . 10^{-5}$ mol dm^{-3}). The reaction mixture was kept at 25°C for 2 h, then oxidized with eight volumes of an oxidizing mixture (30% H_2O_2 : 88% HCOOH = 1 : 9) at 0°C for 30 min (to oxidize the unreacted cysteine

TABLE I

Second-order rate constants (k) for the reactions of 1-(5-R^1-2-furyl)-2-R^2,R^3-ethylenes with thioglycolic acid (R—SH), glycine ($R—NH_2$), phenol (R—OH), and OH^- ions at 25°C

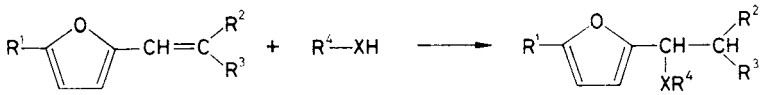
Compound	R^1	R^2	R^3	k, dm^3 mol^{-1} s^{-1}			
				R—SH[a]	$R—NH_2$[b]	R—OH[c]	OH^-[d]
I	NO_2	NO_2	$COOCH_3$	$4·0 . 10^7$	$3·0 . 10^1$	$6·9 . 10^1$	$4·5 . 10^0$
II	$COOCH_3$	NO_2	$COOCH_3$	$1·5 . 10^7$	—	—	$1·9 . 10^1$
III	I	NO_2	$COOCH_3$	$9·6 . 10^6$	$6·4 . 10^1$	$2·1 . 10^2$	$1·2 . 10^1$
IV	Br	NO*a*	$COOCH_3$	$8·2 . 10^6$	$5·1 . 10^1$	$1·2 . 10^2$	$8·1 . 10^0$
V	H	NO_2	$COOCH_3$	$3·9 . 10^6$	$3·0 . 10^1$	$6·7 . 10^1$	$5·8 . 10^0$
VI	CH_3	NO_2	$COOCH_3$	$3·3 . 10^6$	—	—	$3·2 . 10^0$
VII	NO_2	H	NO_2	$5·8 . 10^6$	$8·6 . 10^0$	$1·4 . 10^{-1}$	$2·9 . 10^0$
VIII	H	H	NO_2	$4·2 . 10^5$	$1·8 . 10^0$	$1·8 . 10^{-2}$	$6·8 . 10^{-1}$
IX	NO_2	$COOC_2H_5$	$COOC_2H_5$	$1·8 . 10^5$	$1·2 . 10^0$	$9·6 . 10^{-2}$	$1·8 . 10^{-4}$

pH of medium [a] 5·0; [b] 9·3; [c] 9·0; [d] 12·0.

to cysteic acid). The excess of the oxidizing mixture was distilled off *in vacuo* and the residue was hydrolysed with 10 ml of hydrochloric acid (6 mol dm^{-3}) at 110°C for 22 h. The hydrolysate was taken to dryness *in vacuo*. An experiment without the inhibitor was run in parallel. After evaporation the residues were dissolved in a citrate buffer (pH 2·2) and analysed in an automatic amino acid analyser (AAA Hd 1200, Žiar nad Hronom, Czechoslovakia).

RESULTS AND DISCUSSION

2-Furylethylenes in aqueous media react with —SH, $—NH_2$, and —OH groups of nucleophiles, and with OH^- ions. As shown previously[16,17], the nucleophilic groups attack the C_1 atom of the exocyclic double bond:



Addition-nucleophilic reactions of this type are important in view of strong antimicrobial[7,8] and cytotoxic[9] effects of these compounds. In an attempt to ascertain the biological significance of the individual addition-nucleophilic reactions of 2--furylethylenes, we investigated the affinity of selected derivatives of 2-furylethylene (Table I) to model —SH (thioglycolic acid), $—NH_2$ (glycine), and —OH (phenol) nucleophiles, and to OH^- ions in aqueous media.

The addition-nucleophilic reactions of 2-furylethylenes can be followed spectrophotometrically. These derivatives in aqeous media exhibit a characteristic electron--absorption peak in the region 310–380 nm; the model nucleophiles do not absorb in this region. And since the electron-absorption spectra of the reaction products are significantly different from those of the original 2-furylethylenes, the method can be used to characterize the reactivities of 2-furylethylenes with model nucleophiles. Fig. 1 shows spectrophotometric detection of the reaction of derivative *VI* (Table I) with phenol; the shift of the electron absorption spectrum of the adduct to shorter wave-lengths is characteristic. The kinetic dependence in Fig. 1 (*b*) gives data for calculating the second-order rate constant[15] (naturally with a correction of the first-order rate constant k_{obs} for the reaction of 2-furylethylene with hydroxyl ions of the aqueous medium).

The reaction proceeded in buffered aqueous solutions of pH 5 to 12; the excess of one component, which secured a pseudomonomolecular reaction mechanism, was not higher than twenty-fold. Table I compiles the determined second-order rate constants for reactions of the model 2-furylethylenes with thioglycolic acid (pH of the medium 5·0), glycine (pH 9·3), phenol (pH 9·0), and OH^- ions of an aqueous medium (pH 12). Comparison of the reactivities (Table I) shows the prominent place of —SH compounds in the addition-nucleophilic reactions of 2-furylethylenes. The second--order rate constants for the reactions of 2-furylethylenes with the thiol anion are

5 to 7 orders of magnitude higher than for the reactions with the amino or hydroxy group. This difference is more visible on the first-order rate constants, k_{obs} (s^{-1}). Thus, in a neutral aqueous solution with an equal concentration of all the nucleophiles, the first-order rate constants, k_{obs}, for the reactions of the derivative *I* with thioglycolic acid, glycine and phenol would be in a ratio of $10^6 : 2 : 3$. The reactivity of a 2-furylethylene with the nucleophilic groups studied naturally also depends on the nature of the functional group in the 2-position of the exocyclic double bond and the 5-position of the furan ring, and increases with their electron-withdrawing effect. This type of addition-nucleophilic reactions of 2-furylethylenes is also influenced by the nature of the nucleophile. As has been shown before[18,17], the second order rate constants for the reactions of 2-furylethylenes with low-molecular-mass thiols and amines increase (by as many as 5 orders of magnitude) with both the increase of the dissociation constants and the increase in basicity of the nucleophiles.

These facts might help in seeking an answer to the question which thiol or amino compounds will react by the addition-nucleophilic mechanism with 2-furylethylenes preferentially. The avidity of 2-furylethylene for low-molecular-mass thiols *in vitro*

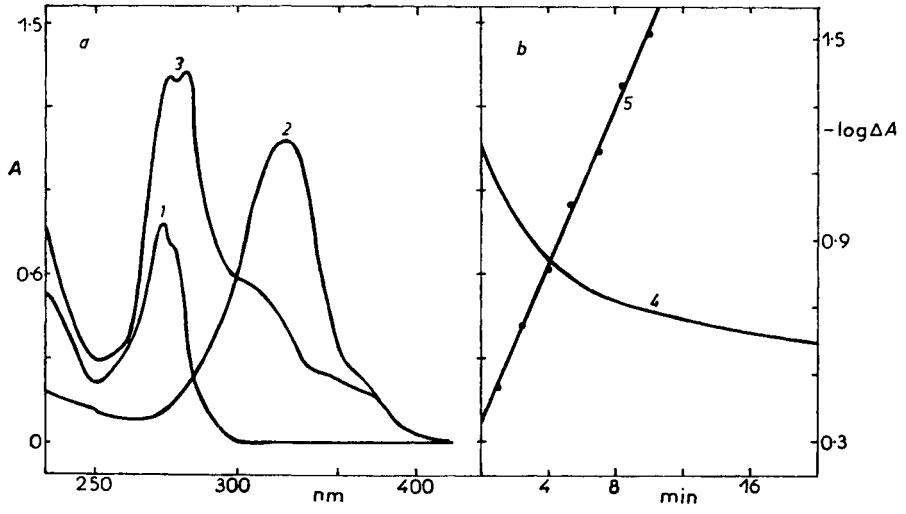


FIG. 1

Electron absorption spectra (*a*) of phenol (*1*), 1-(2-furyl)-2-nitro-2-methoxycarbonylethylene (*2*), their final reaction mixture (*3*) and the kinetics of this reaction (*b*) monitored at 320 nm; plots of A_t (*4*) and log A (*5*) *vs* time. The initial concentrations of 2-furylethylene and phenol were $5 . 10^{-5}$ mol dm^{-3} and $5 . 10^{-4}$ mol dm^{-3}, respectively. The spectra and the kinetics were measured in Clark and Lub's buffer of pH 9·0 at 25°C in 1-cm cells. $\Delta A = A_t - A_{t+\Delta t}$, $\Delta t =$ = 4 min

somewhat overshadows the possible attack of OH^- ions, followed by hydrolysis, giving rise to the furfuryl aldehyde grouping (highly active biologically)[17].

A prominent position of low-molecular-mass thiols in the addition-nucleophilic reactions of 2-furylethylenes is obvious. However, the antimicrobial and cytotoxic efficacy of these substances is due mainly to their ability to modify various enzymes of the energetic metabolism and other biopolymers[17,19]. The functional groups of biomacromolecules reacting with 2-furylethylenes were determined by amino acid analyses of the modified and non-modified proteins. Alcohol dehydrogenase was allowed to react with the derivative *I* for 2 h in an aqueous medium containing bovine serum albumin. The content of cysteine in the modified alcohol dehydrogenase was more than 30% lower (Fig. 2), but the contents of the other amino acids were not much changed either in the dehydrogenase or in any of the other proteins studied. The derivative *I* modified only the SH group of cysteine; the addition product was seen on the analytical record between cysteic acid and aspartic acid. The other nucleophilic groups of the protein side chains, such as NH_2 (lysine, N-terminal amino acids) or OH (serine, threonine, tyrosine) were not affected to a significant extent. In a previous study[19], describing reaction of the derivative *I* with glyceraldehyde-3-phosphate dehydrogenase, the results were analogous. The findings confirm the preferential role of SH groups in addition-nucleophilic reactions of 2-furylethylenes on the exocyclic double bond. The biological impact of this reaction

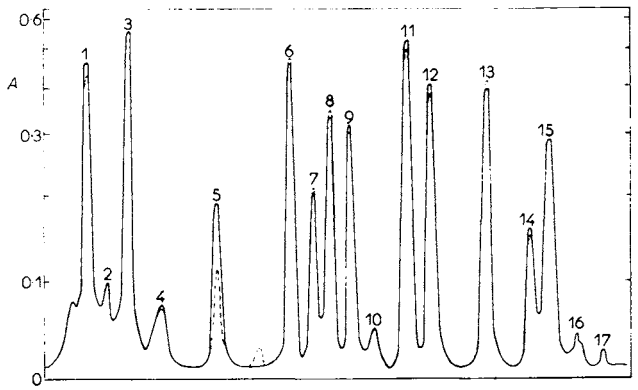


FIG. 2

Amino acid contents of alcohol dehydrogenase before (solid line) and after (dashed line) its reaction with 1-(5-nitro-2-furyl)-2-nitro-2-methoxycarbonylethylene (*I*). The dehydrogenase (2 mg/cm^3) was allowed to react with the inhibitor ($1 . 10^{-5}$ mol dm^{-3}) for 2 h at 25°C. The amino acid composition of the hydrolysate was determined by an automatic analyser as: **1** Lys, **2** His, **3** NH_4. **4** Arg, **5** $CYSO_3H$, **6** Asp, **7** Thr, **8** Ser, **9** Glu, **10** Pro, **11** Gly, **12** Ala, **13** Val, **14** Ileu, **15** Leu, **16** Tyr, **17** Phe

consists mainly in modification of the low-molecular-mass SH precursors and thiol enzymes, resulting in strong antimicrobial and cytotoxic effects of 2-furylethylenes.

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Translated by J. Salák.