# SUBSTITUTION REACTIONS OF SOME (5-NITRO-2-FURYL)ETHYLENE DERIVATIVES WITH THIOLS*

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1-(5-Nitro-2-furyl)-2-X-ethylenes ($X = Br, \overset{(+)}{N}(CH_3)_3\overset{(-)}{Br}, SO_2Ph$) undergo substitution with 2-mercaptoacetic acid in aqueous medium to give S-(2-(5-nitro-2-furyl)ethenyl)mercaptoacetic acid which is decomposed in excess 2-mercaptoacetic acid to give unstable compounds which could not be identified. The rate constants of both consecutive reactions have been determined spectrophotometrically in a buffer of pH 5·0 at 25°C.

The derivatives of (5-nitro-2-furyl)ethylene are biologically active compounds with a broad spectrum of antimicrobial, cytotoxic, cancerostatic, but also carcinogenic and mutagenic effects[1,2]. Whereas the mutagenic properties of these compounds seem to be connected with the presence of the 5-nitro group in the furane ring[3-5], for their antimicrobial and cytotoxic activity the essential property is their reactivity with nucleophilic components of a cell especially with the catalytically active thiol-proteins[6]. Model studies of the reactions of (5-nitro-2-furyl)ethylene derivatives with low-molecular weight thiols showed that, in most cases studied, the thiol is added to the exocyclic double bond[7]. Analysis of the products and kinetic characterization of analogous reactions in the cases where no addition takes place in the first step are dealt with in the present paper.

## EXPERIMENTAL

*Reagents.* The 1-(5-nitro-2-furyl)-2-X-ethylenes were synthetized according to known procedures ($X = Br$, *Ia*)[8], ($X = N(CH_3)_3Br$, *Ib*)[9], ($X = SO_2Ph$, *Ic*)[10]. The product of the substitution reactions of these compounds with 2-mercaptoacetic acid, S-(2-(5-nitro-2-furyl)ethenyl)mercaptoacetic acid (*II*), was synthetised in the following ways:

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*a*) From (*Z*)-1-(5-nitro-2-furyl)-2-bromoethylene (*Ia*). 2·18 g (0·01 mol) (*Z*)-*Ia* was dissolved in 50 ml 1,4-dioxane and mixed with 1 g thioglycollic acid and 0·85 g NaOH in 25 ml water. After 24 h standing at room temperature, the reaction mixture was cooled to 5°C and acidified with HCl (1 mol $dm^{-3}$). The solvent was distilled off in vacuum, and the residue was purified by means of column chromatography (silica gel 1 . $10^{-2}$ mm; benzene–ethyl acetate). Yield: 0·8 g (35%) (*E*)-*II*, m.p. 135–137°C and 0·6 g (26%) (*Z*)-*II*, m.p. 165–167°C. For $C_8H_7NO_5S$ (229·2) calculated: 6·11% N, 13·99% S; found: 6·21 and 6·17% N, 13·72 and 13·80% S for the (*E*)- and (*Z*)-isomers, resp. $^1$H NMR spectrum (hexadeuteriodimethyl sulphoxide): (*E*)-*II*: 7·63 (d, $J$ = 4·0 Hz, 1 H, $C_{fur}$—$H_4$); 6·71 (d, $J$ = 4·0 Hz, 1 H, $C_{fur}$—$H_3$); 7·41 (d, $J$ = 16·0 Hz, 1 H, $CH_A$=); 6·47 (d, $J$ = 16·0 Hz, 1 H, $CH_B$=); 3·81 (s, 2 H, —$CH_2$—). (*Z*)-*II*: 7·68 (d, $J$ = 4·0 Hz, 1 H, $C_{fur}$—$H_4$); 6·12 (d, $J$ = 4·0 Hz, 1 H, $C_{fur}$—$H_3$); 7·03 (d, $J$ = 11 Hz, 1 H, $CH_A$=); 6·53 (d, $J$ = 11 Hz, 1 H, $CH_B$=); 3·77 (s, 2 H, —$CH_2$—).

*b*) From (*Z*)-2-(5-nitro-2-furyl)ethenyltrimethylammonium bromide (*Ib*). 5·5 g (0·02 mol) (*Z*)-*Ib* was dissolved in 100 ml water, and a solution of 1·85 g thioglycollic acid and 1·6 g NaOH in 20 ml water was added thereto within 10 min. After 1 h at room temperature and 1 h boiling, the reaction mixture was cooled to 5°C and acidified with HCl (1 mol $dm^{-3}$). The orange-brown crystalline precipitate was extracted with dichloromethane. After distilling off the solvent, the residue was purified by means of column chromatography (silica gel as *sub a*). Yield: 2·8 g (61%) (*E*)-*II*, m.p. 135–137°C (orange crystals) and 0·77 g (17%) (*Z*)-*II*, m.p. 165–167°C.

*c*) From (*E*)-1-(5-nitro-2-furyl)-2-phenylsulphonylethylene (*Ic*). 2·8 g (0·01 mol) (*E*)-*Ic* was dissloved in 30 ml 1,4-dioxane and mixed with 1 g thioglycollic acid and 0·85 g NaOH in 15 ml $H_2O$. After 24 h standing at room temperature, the reaction mixture was cooled to 5°C and acidified with HCl (1 mol $dm^{-3}$). The solvent was distilled off in vacuum, and the residue was purified by means of columns chromatography (silica gel as *sub a*). Yield: 0·7 g (31%) (*Z*)-*II*, m.p. 165–167°C, and 1·1 g (48%) (*E*)-*II*, m.p. 135–137°C. All the furylethylenes mentioned (*Ia*, *Ib*, *Ic*, *II*) were dissolved in 1,4-dioxane (freshly distilled with sodium metal), and the stock solutions were used in the below-described kinetic experiments.

*The* UV-VIS *spectra* of the compounds studied and of their reaction mixtures with 2-mercaptoacetic acid were measured with a Specord UV VIS apparatus (Zeiss, Jena) in 0·2 mol $dm^{-3}$ buffers according to Clark and Lubs (pH 5·0)[11] with 0·5 vol. % dioxane in the range of 250 to 600 nm.

*The kinetic measurements* were carried out with a UV-VIS spectrophotometer SP 30 (Pye Unicam, Cambridge) at 440 nm. The reaction mixtures of 100 $cm^3$ total volume (the Clark and Lubs[11] buffer solution pH 5·0 (0·2 mol $dm^{-3}$), the 2-furylethylene examined, *Ia*, *Ib*, *Ic*, *II* (5 . . $10^{-5}$ mol $dm^{-3}$), 2-mercaptoacetic acid (1 to 9 . $10^{-3}$ mol $dm^{-3}$), and 0·5 vol. % dioxane) were incubated at (25 ± 0·1)°C with exclusion of light. At suitable time intervals, 2 ml samples were taken from the reaction mixture for the spectrophotometric measurements. After the measurement, the samples were not returned to the reaction mixture. The pH values of the reaction mixture were checked by means of a pH-meter OP 208/1 (Radelkis, Budapest) during the reaction. With an at least twentyfold excess of 2-mercaptoacetic acid, the concentration of ionized thiol could be considered constant throughout the experiment.

The rate constant of the reaction of acid *II* with 2-mercaptoacetic acid was determined according to ref.[12] from the slope of the linear time dependence of $\ln |A_t - A_{t+\Delta t}|$:

$$\ln |A_t - A_{t+\Delta t}| = \ln |(A_0 - A_\infty)(1 - \exp(-kc\,\Delta t))| - kct\,, \qquad (1)$$

where $A$ means the absorbance at the time indicated by the subscript, $\Delta t$ means the suitably

chosen time interval, $k$ ($dm^3\ mol^{-1}\ s^{-1}$) is the rate constant, $c$ stands for the initial concentration of the ionized thiol calculated with the use of known[13] $pK_a = 10{\cdot}22$.

The rate constants of the reactions of the furylethylenes *Ia*, *Ib*, *Ic* were determined from the relations describing the kinetics of consecutive reactions of the first order[12] under the presumption that the concentration of the ionized thiol does not change throughout the reaction:

$$t_{max} = \ln (k_1/k_2)/(k_1 - k_2)c \qquad (2)$$

and

$$c_{max} = c_0(k_2/k_1)^{k_2/(k_1-k_2)}, \qquad (3)$$

where the subscript max indicates the relation to the maximum concentration of the intermediate *II*, $c_0$ means the initial concentration of the furylethylene studied, and $c$ stands for the initial concentration of the ionized thiol.

The rate constants given are mean values from three to four measurements each, the reproducibility error being about 5%. The accuracy of the determination was verified by simulating the kinetic curves according to the respective relations[12].

## RESULTS AND DISCUSSION

The course of the reactions of (5-nitro-2-furyl)ethylene derivatives with 2-mercaptoacetic acid in dilute aqueous solutions can easily be followed by means of spectrophotometry (Fig. 1). The starting compounds *Ia*, *Ib*, and *Ic* have their absorption maxima from 340 nm to 360 nm, whereas 2-mercaptoacetic acid does not absorb in this region. In the reaction course an intermediate is formed first (the absorption maximum about 430 nm) which is then transformed into compounds absorbing below 390 nm. The reactions on preparative scale in water (with addition of 1,4-dioxane, in the case of *Ia* and *Ic*, to increase the solubility) gave 60–80% yields of mixtures of (Z)-*II* and (*E*)-*II*. This product has the same UV-VIS spectrum as the intermediate (with the maximum at 430 nm). No other products could be identified even in the cases of the experiments starting from the intermediate *II*. Also unsuc-

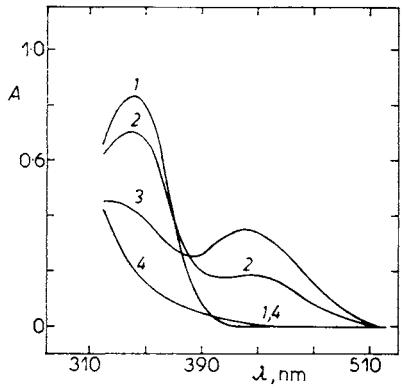
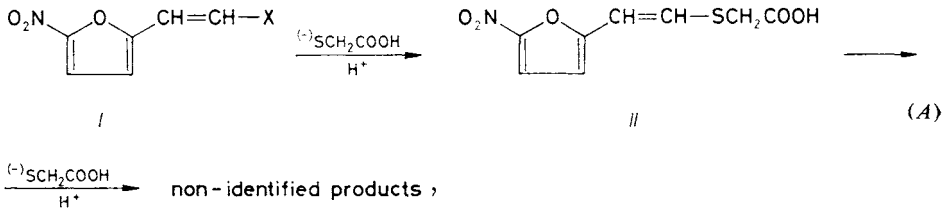



FIG. 1

The UV-VIS absorption spectra of compound (*Z*)-*Ib* ($5 . 10^{-5}$ mol $dm^{-3}$; *1*) and of its reaction mixture with 2-mercaptoacetic acid ($3 . 10^{-3}$ mol $dm^{-3}$) after 3 (*2*), 10 (*3*), and 100 h (*4*) incubation in a Clark–Lubs buffer[11] of pH 5·0 at 25°C

cessful was the attempt at the estimation of structure of the final product by means of $^1H$ and $^{13}C$ NMR spectroscopy directly applied to the reaction mixture (without isolation of the components). The kinetic analysis showed that one molecule of 2-mercaptoacetic acid takes part in each of the two consecutive steps. The reaction of compounds *Ia*, *Ib*, and *Ic* with this thiol can be described by the following scheme:




Considering the importance of reactivity of the compounds studied (*Ia*, *Ib*, *Ic*) for their biological activity, we should, first of all, investigate the rate constant of the first reaction, because the activity consists in modifications of nucleophilic groups in biopolymers. Nevertheless, we also determined the rate constant of the second reaction, because its knowledge considerably simplifies the kinetic analysis.

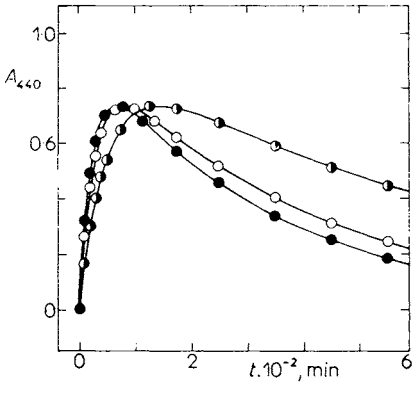



FIG. 2

The absorbance–time dependence of S-(2-(5-nitro-2-furyl)ethenyl)mercaptoacetic acid during the reaction of (*E*)-*Ic* ($5 . 10^{-5}$ mol . $dm^{-3}$) with 2-mercaptoacetic acid. The concentrations of the ionized thiol: $1{\cdot}80 . 10^{-8}$ (◑), $3{\cdot}22 . 10^{-8}$ (○), and $3{\cdot}84 . 10^{-8}$ mol $dm^{-3}$ (●). The Clark–Lubs buffer[11] of pH 5·0 at 25°C

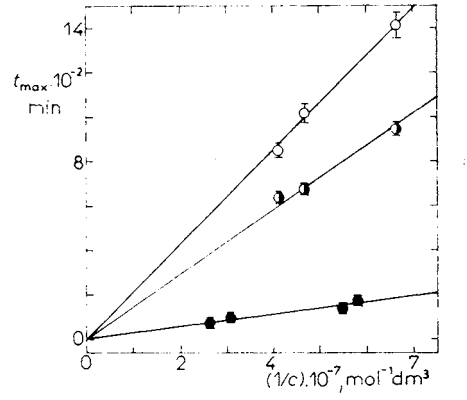



FIG. 3

The dependence of $t_{max}$ (the time needed for reaching the maximum concentration of the intermediate *II*) on reciprocal value of the ionized thiol concentration for the reactions of 2-mercaptoacetic acid with *Ia* (◑), *Ib* (○), and *Ic* (●) in a Clark–Lubs buffer[11] of pH 5·0 at 25°C

TABLE I

The rate constants $k$ ($dm^3\ mol^{-1}\ s^{-1}$) of the reactions of *Ia*, *Ib*, *Ic*, and *II* with 2-mercaptoacetic acid in a Clark–Lubs buffer[11] of pH 5·0 at 25°C

| *Ia* | *Ib* | *Ic* | *II* |
|---|---|---|---|
| $(9{\cdot}7 \pm 0{\cdot}5) \cdot 10^2$ | $(1{\cdot}3 \pm 0{\cdot}1) \cdot 10^3$ | $(1{\cdot}8 \pm 0{\cdot}1) \cdot 10^4$ | $(1{\cdot}3 \pm 0{\cdot}1) \cdot 10^3$ |

The kinetics of the reactions of compounds *Ia*, *Ib*, and *Ic* with 2-mercaptoacetic acid was followed spectrophotometrically at 440 nm in a buffer of pH 5·0 at 25°C in the presence of an at least twentyfold excess of the thiol which participates in the reaction as the ionized species[13]. The concentration of the ionized thiol does not practically change throughout the reactions, hence their course is pseudomonomolecular. In the case of compounds *Ia*, *Ib*, and *Ic*, the time-concentration dependence of the intermediate *II* is followed at 440 nm (Fig. 2). It can be seen that the maximum concentration of *II* does not depend on the thiol concentration. On the other hand, the time $t_{max}$ necessary for obtaining the maximum concentration of *II* depends linearly on the reciprocal value of the ionized thiol concentration (Fig. 3). Both these facts agree with the relations valid for kinetics of consecutive reactions[12]. The way of determination of the individual rate constants is given in Experimental, and the results are summarized in Table I.

The rate constants of the reaction of compounds *Ia*, *Ib*, and *Ic* with 2-mercaptoacetic acid were determined at the same conditions as those of the addition reactions of 2-furylethylene with this thiol[14]. This fact makes it possible to include the derivatives studied (*Ia*, *Ib*, *Ic*) into the common series with other 2-furylethylenes, which was compiled with the aim of studies of the effect of reactivity to nucleophiles on the biological activity of these compounds. The deviation from expected values of biological activity in the case of compounds *Ia*, *Ib*, and *Ic* could indicate a modification of bioological activity of these compounds due to release of the original substituent X in biological material.

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