BIOCHEMICALLY IMPORTANT REACTIONS OF 2-FURYLETHYLENES. REACTIONS WITH LOW-MOLECULAR THIOLS

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As evidenced by spectrophotometry and radiochromatography, derivatives of 2-furylethylene are able to react with thiols in an aqueous medium. The NMR analysis of reaction mixture showed that the site attacked by the thiol is the more electrophilic $C_{(1)}$ atom of the exocyclic double bond of the 2-furylethylenes under study. The ability to react with SH groups is of extraordinary importance particularly in connection with the study of mechanism of biological activity of 2-furylethylenes.

2-Furylethylenes have been used as antimicrobial substances in a clinical or veterinary medicine, as preservatives in food industry and as growth stimulators for farm animals[1-5]. Findings that some derivatives possess mutagenic and carcinogenic activities[6-10] are somehow disappointing. The mechanism of antimicrobial[11,12], and also cytotoxic effects of 2-furylethylenes towards animal cells[13] lies in the exclusion of energy-yielding processes, *i.e.* of glycolysis and oxidative phosphorylation. This effect is due to an inhibition of thiol enzymes of the already mentioned pathways. Hexokinase (EC 2.7.1.1), phosphofructokinase (EC 2.7.1.11) and glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) are those responsible for glycolysis inhibition. The inactivation of the afore mentioned enzymes is caused by a chemical modification of their catalytically active sulfhydryl groups[14,15]. This paper is aimed to investigate the biochemically important nucleophilic reactions of 2-furylethylenes with low-molecular thiols.

EXPERIMENTAL

All furylethylenes under investigation (Table I) were kindly donated by Professor J. Kováč, Department of Organic Chemistry, Slovak Institute of Technology, Bratislava. The reduced glutathione (GSH, γ-L-glutamyl-L-cysteinylglycine) and 2-aminoethanethiol . HCl (Cysteamine . . HCl) were Sigma (USA) products. ^{35}S-Benzylmercaptan was synthesized by a procedure described in[16,17] (specific radioactivity $1 . 10^8$ Bq mmol^{-1}). The radiochemical purity of the product determined after reaction with $AgNO_3$ was found to be 97% (autoradiographic detection, Silufol Kavalier, Czechoslovakia, solvent ethanol). The purity of thiols was estimated spectro-

photometrically with Ellman reagent[18] (Fluka, Switzerland). Deuteriated water (Techsnabexport USSR) was employed for preparation of some samples for NMR analysis. The spectrophotometric indication of reactions, the UV and visible spectra of 2-furylethylenes and their mixtures with glutathione were measured in Clark–Lubs buffer solutions[19] of pH 4–8 at 25°C and initial concentrations of 2-furylethylene $5 \cdot 10^{-5}$ mol l^{-1} and GSH $1 \cdot 10^{-3}$ mol l^{-1}. 2-Furylethylenes were added to the reaction mixtures from stock solutions in methanol. The final concentration of methanol in reaction mixtures was 1%. The UV spectra were recorded with a Specord UV-VIS spectrophotometer (Zeiss, Jena).

Radiochromatographic proof: The mixture containing ^{35}S-benzylmercaptan ($5 \cdot 10^{-2}$ mol l^{-1}) and the appropriate 2-furylethylene ($1 \cdot 10^{-2}$ mol l^{-1}) was chromatographically separated after a 20-h incubation at room temperature on Silufol UV 254 plates using benzene as solvent. Impulse counter Tesla NZQ 612 (Czechoslovakia) with a window GM tube Tesla, type 30/30 AB was employed for radiochromatographic analysis.

NMR *analysis*: 2-Furylethylenes were reacted with 2-aminoethanethiol in water (2 ml) at a molar ratio 1 : 1 to 1 : 4 (2-furylethylene : RSH); the final concentration of 2-furylethylenes was at least 0·1 mol l^{-1}. In most cases the reaction mixture was heated up to 60°C in order to dissolve 2-furylethylenes. All NMR spectra were taken with a Jeol FX-100 (Japan) spectrometer equipped with a multinuclear probe, operating at 99·61 MHz for ^{1}H and 25·05 MHz for ^{13}C nuclei. Three types of ^{13}C NMR spectra were measured: normal proton-noise-decoupled spectra, off resonance proton-decoupled spectra, and single resonance spectra[20]. Internal reference substance for ^{13}C NMR spectroscopy was dioxane ($\delta = 67{\cdot}0$); spectral width 6 002 Hz.

RESULTS AND DISCUSSION

2-Furylethylenes react with low-molecular thiols. This was found by investigation of their reactivity towards glutathione, which was the model thiol in these experiments. The free SH group of the cysteine embodied in glutathione (γ-glutamyl-L--cysteinylglycine) can in more "chemical" experiments suitably imitate the SH groups of the embodied cysteine in catalytically or structurally important thiol-proteins[21].

Reactions of 2-furylethylenes with glutathione can be investigated by spectrophotometry. 2-Furylethylenes have noticeable absorption maxima in the 300–500 nm range. Aliphatic thiols, on the other hand, do not absorb in this spectral region and therefore, these reactions can be spectrophotometrically monitored since the electronic absorption spectra of reaction products significantly differ from those of 2-furylethylenes themselves (Fig. 1). This observation is usable for characterization of reactivities of 2-furylethylenes with thiols[22].

A more exact view on the reaction products of 2-furylethylenes with thiols offered radiochromatography employing ^{35}S-benzylmercaptan as model thiol. Fig. 2 shows that the reaction of 2-furylethylenes *I*, *III*, *VI* with radioactive benzylmercaptan affords always one reaction product. Unfortunately enough, benzylmercaptan is quite volatile and therefore, the corresponding reactions cannot be quantitatively evaluated. Derivatives *IV*, *VI* and *VII* were reacted with a radioinactive benzylmercaptan and the structure of reaction products was elucidated[15]. All isolates were

addition products having the thiol attached to $C_{(1)}$ of the exocyclic double bond of 2-furylethylenes.

Since benzylmercaptan is highly insoluble in aqueous systems, the reactions were carried out in methanol; it was, however interesting to know, whether the above-mentioned addition products are typical for the reaction of 2-furylethylenes with thiols in aqueous solutions, as well. Therefore, the aqueous reaction mixtures of the corresponding 2-furylethylenes with 2-aminoethanethiol hydrochloride were analyzed by NMR spectrometry. This thiol was selected because of its very good solubility in water. Consequently, an increased solubility of the addition products could be anticipated, this being very important for the NMR measurement. Reactions were carried out in water or deuteriated water in a 2-furylethylene to RSH ratio 1 : 1 to 1 : 4.

The ^{1}H MNR spectra of reaction mixtures reveal in the region corresponding to aromatic and olefinic protons only the signal of protons of the furan ring. The signal associated with protons of vinylic group was absent, thus evidencing that an addition of the thiol to the double bond took place. Due to a weak spectral resolution it was impossible to ascertain the mode of thiol addition from the ^{1}H NMR spectra. The ^{13}C NMR data afforded more information necessary for the complete structure determination of products.

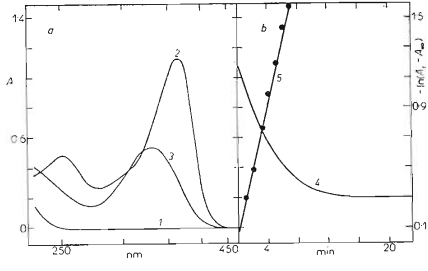


FIG. 1

Electron absorption spectra; *a* of glutathione (*1*), 1-(5-nitro-2-furyl)-2-nitroethylene (*2*), their reaction mixture (*3*) and kinetics of the reaction *b* examined at 365 nm (the dependence A_t (*4*) and $\ln(A_t - A_\infty)$ (*5*) *versus* time). The initial concentrations of GSH $1 \cdot 10^{-3}$ mol l^{-1}, of 2-furylethylene $5 \cdot 10^{-5}$ mol l^{-1}, Clark–Lubs buffer[19] of pH 4·1, temperature 25°C. A_t, A_∞ absorbances in time t and after the reaction. Cell width 1 cm

The number and position of signals in the normal carbon spectra in the sp^2-hybridized carbon regions evidenced that the reaction proceeds as an addition of the thiol to the vinyl group. This addition reaction can proceed in four ways: the vinyl

TABLE I

1-(5-R^1-2-Furyl)-2-R^2,R^3-ethylenes and ^{13}C NMR chemical shifts of products resulting from the reaction with 2-aminoethanthiol

Compound	R^1	R^2	R^3	$C_{(1)}$	$C_{(2)}$	$S—CH_2$	$N—CH_2$
I[a]	H	NO_2	$COOCH_3$	42·1	88·5	28·8	38·6
				43·3	87·6	28·1	
II[a]	Br	NO_2	$COOCH_3$	42·0	88·6	28·6	38·6
				41·3	87·7	28·0	
III	NO_2	NO_2	$COOCH_3$	—	—	—	—
IV	NO_2	$COOC_2H_5$	$COOC_2H_5$	—	—	—	—
V	NO_2	COOH	H	40·0	75·7	28·4	39·4
VI	NO_2	NO_2	H	48·8	31·7	28·8	39·3
VII	H	NO_2	H	39·5	76·7	28·1	39·4

[a] Product is the mixture of diastereoisomers.

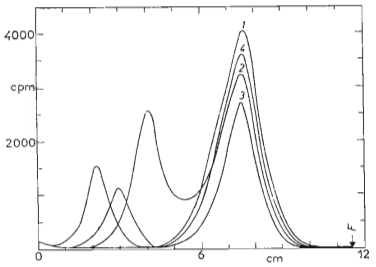
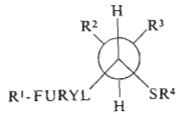


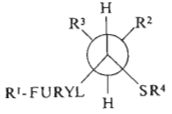
FIG. 2

Radiochromatogram of ^{35}S-benzylmercaptan (*1*) and its reaction mixture with 1-(2-furyl)-2--nitro-2-methoxycarbonylethylene (*2*), 1-(5-nitro-2-furyl)-2-nitro-2-methoxycarbonylethylene (*3*), 1-(5-nitro-2-furyl)-2-nitroethylene (*4*) in methanol after a 20 h-incubation at room temperature. The initial concentrations of the thiol $5 . 10^{-2}$ mol l^{-1}, of 2-furylethylenes $1 . 10^{-2}$ mol l^{-1}. Separated on Silufol plates, solvent benzene

group reacts with the thiol or amino group of 2-aminoethanethiol hydrochloride either at $C_{(1)}$, or $C_{(2)}$ of the exocyclic double bond. The attack of the more electrophilic carbon of the vinyl group by the nucleophilic SH group is much more probable, since the amino group is in a protonized form ($-NH_3^+$) in the given medium, so that its nucleophility is virtually negligible when compared with the mercapto group. The position of addition of 2-mercaptoethylamine hydrochloride in derivatives of 2-furylethylene having R^2 and R^3 different from hydrogen can be determined from the number of signals in the spectrum. If the more reactive group is bound to $C_{(1)}$ and hydrogen to $C_{(2)}$ then the reaction product should have two chiral centers and should be a mixture of both diastereoisomers; these (*a* and *b*) are discernible



a



b

in the ^{13}C NMR spectrum. In the reverse case a mixture of enantiomers, which cannot be differentiated by NMR method would be formed. The number and position of signals (Table I) in the spectra of these derivatives (*I* and *II*) really indicate that the product is a mixture of two diastereoisomers, *i.e.* that 2-aminoethanethiol hydrochloride was attached at $C_{(1)}$. This fact also is unequivocally proved by the off resonance multiplicity of $C_{(1)}$ and $C_{(2)}$ signals. Both signals were seen in the off resonance spectrum as a doublet, what means that each of them has a directly bound hydrogen atom.

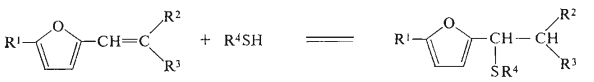
The product resulting from derivatives with $R^2 = H$ cannot be a mixture of diastereoisomers. The off resonance multiplicities offer a structural information providing $C_{(1)}$ and $C_{(2)}$ could be differentiated on the basis of their chemical shift. This is the case with derivatives with $R^2 = NO_2$ (derivatives *VI* and *VII*, Table I); due to a strong polar effect of the nitro group the chemical shift of $C_{(2)}$ is greater than that of $C_{(1)}$. As it follows from the off resonance spectra of these derivatives, two directly bonded hydrogen atoms are at $C_{(2)}$ *i.e.* 2-aminoethanethiol hydrochloride is attached to $C_{(1)}$.

The single resonance spectrum was needed for determination of the site of addition of 2-mercaptoethylamine hydrochloride in derivative *V*, since it maintains the spin–spin interaction with protons. Under a reasonable presumption that the long-range interactions with protons of the furan ring will not be seen in position 2, the multiplet of the $C_{(2)}$ should be less complicated by small interactions as that of $C_{(1)}$. The inspection of signals belonging to $C_{(1)}$ and $C_{(2)}$ showed that the CH_2 signal (triplet,

δ 31·7, ^1J (H, c) = 133 Hz) is complicated by one small interaction, only. On the other hand, the pattern of the CH (doublet, δ 31·7, ^1J (H, c) = 143 Hz) reveals that this carbon is in a spin–spin interaction with protons of the furan ring. As it follows, the CH is in position 1, *i.e.* the addition took place as in preceding cases, namely at $C_{(1)}$.

The values of chemical shifts of $C_{(1)}$ (c. 40 ppm) evidenced that 2-aminoethanethiol hydrochloride is attached to 2-furylethylenes through sulfur. Should it be linked through the more electronegative nitrogen, the chemical shift of $C_{(1)}$ will be by 10 to 20 ppm greater. The difference in chemical shift of $-CH_2$-S-carbon is greater (c. 0·6 ppm) than that of carbons -NH-CH_2- (0 ppm) with products, which are the mixture of two diastereoisomers. This proves that -S-CH_2- is closer to the center of chirality than -NH-CH_2-, *i.e.* aminoethanethiol hydrochloride is bonded through sulfur in this product. Uncertain is the bond of thiolamine in derivative *V* (R^2 = = COOH). The chemical shift of $C_{(1)}$ is too high (δ 48·8), what can be due to addition through nitrogen. In connection with this finding is worth noting that the reaction medium in this very experiment had to be alkaline (pH c. 11; as a consequence, the nucleophility of nitrogen of 2-aminoethanethiol hydrochloride was considerably enhanced; transition of $-NH_3^{(+)}$ to $-NH_2$, p*K*a = 8·39, ref.[19]).

It can be concluded that the reaction of the above-mentioned 2-furylethylenes with thiols proceeds as follows:



The attacked site of the nucleophillic SH group of the respective thiol is, therefore, $C_{(1)}$ of the exocyclic double bond. It is, however, not excluded that an appropriate change of substituents could cause the transfer of the reaction site to the other carbon of the unsaturated double bond, or alternatively, to the furan ring. Results of the quantum-chemical study of 2-furylethylenes[23] favour this possibility.

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