REACTIONS OF 1-(5-NITRO-2-FURYL)-2-NITROETHYLENE WITH AMINO AND HYDROXY GROUPS*

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Reactions have been studied of 1-(5-nitro-2-furyl)-2-nitroethylene with $-NH_2$ and $-OH$ groups of low-molecular compounds (butylamine, aniline, glycine, taurine, glucosamine, tyramine, tryptamine, noradrenaline, histamine, ethanol, methanol, OH^- ions) as well as biopolymers (ribonuclease, albumin DNA, RNA, Newcastle disease virus). With the low-molecular amines and alcohols it has been found that the reactions proceed as nucleophilic additions in aqueous medium, and the respective nucleophilic groups attack the more electrophilic $C_{(1)}$ atom of the exocyclic double bond of nitrofurylethylene. The modifications proved with the above-mentioned biopolymers *in vitro* indicate a possibility of direct interaction (without metabolic activation) between 5-nitro-2-furylethylenes and proteins or nucleic acids *in vivo*. These findings are significant from the point of view of recognizing mutagenic effect of nitrofurylethylenes and general biotoxicity of these compounds.

5-Nitro-2-furylethylenes were (and some of them still are) used as antimicrobial substances in clinical and veterinary medicine, as conservation agents in foodstuff industry, or as growth stimulants for domestic animals[1-5]. Antimicrobial[6,7] as well as cytotoxic effect of 5-nitro-2-furylethylenes on animal cells[8] is due to elimination of the energy supplying processes, *i.e.* glycolysis and oxidative phosphorylation. The ability of these compounds to modify the catalytically active thiol groups of the corresponding enzymes of the said metabolic path is caused by nucleophilic addition reactions at the exocyclic double bond of the nitrofurylethylenes with thiolate anions as nucleophilic reagents (see our previous communications[9-12]). Besides that still other nucleophilic addition reactions of these compounds (expecially with biopolymers containing amino and hydroxyl groups) could be important with respect to high mutagenic and carcinogenic activity found with some 5-nitro-2-furylethylene

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derivatives[13-17]. Therefore, the aim of the present communication is a study of biochemically interesting nucleophilic reactions of 1-(5-nitro-2-furyl)-2-nitroethylene (as a representative of 5-nitro-2-furylethylenes) with low-molecular amines and alcohol as well as with biopolymers of non-thiol type.

EXPERIMENTAL

Chemicals and biopolymers: 1-(5-Nitro-2-furyl)-2-nitroethylene was synthetized by the procedure given in ref.[18], coenzym A, dihydrolipoic acid, and the low-molecular biologically active amines were products of the firm Sigma (St. Louis, U.S.A.). Human serum albumine (Dade, U.S.A.). Ribonuclease from bovine pancreas (E3 2.7.7.16) (Reanal, Hungary). Pyronin G and the yeast RNA and DNA isolated from calf thymus were products of the firm Fluka (Switzerland). Newcastle disease virus, Kansas strain was propagated in the allantoic cavity of embryonated eggs (Institute of Virology, Slovak Academy of Sciences, Bratislava). The infectious allantoic fluid was kept at 4°C.

The UV and VIS spectra of 1-(5-nitro-2-furyl)-2-nitroethylene and its reaction products with amines, alcohols, proteins, and nucleic acids as well as the reaction kinetics were measured with a Specord UV VIS spectrophotometer (Zeiss, GDR) in 0·2 mol l^{-1} buffer solutions according to Clark & Lubs (pH 6 to 10, ref.[19]) at 25°C. The solutions were adjusted by means of a pH-Meter OP 207 (Radelkis, Hungary). The stock solutions of 1-(5-nitro-2-furyl)-2-nitroethylene in dimethyl sulphoxide were prepared in such way that the reaction mixture contained at most 0·5% (v/v) of this solvent. The initial concentration of 1-(5-nitro-2-furyl)-2-nitroethylene in the kinetic measurements was $5 \cdot 10^{-5}$ mol l^{-1}, the amines and alcohols were applied in the concentrations of $1 \cdot 10^{-1}$ to $5 \cdot 10^{-3}$ mol l^{-1}. At these conditions the nucleophile to substrate concentration ratio was at least 20 : 1, and the reactions took pseudomonomolecular course. The kinetic measurements were carried out at 365 nm in the region of absorption maxima of the derivative studied, where the other reactants (thiols, amines, alcohols) did not absorb. The second order rate constant was calculated from the relation[20]

$$k = k_{obs}(K_a + c_{H^+})\, c_0^{-1} K_a^{-1} ,$$

where k_{obs} means the first order rate constant, K_a is the dissociation constant of the nucleophilic reagent, c_0 (mol l^{-1}) is the initial analytical concentration, and c_{H^+} (mol l^{-1}) means the proton concentration. The first order rate constants were obtained from the relation

$$k_{obs} = 2{\cdot}3(\log (A_{t_2} - A_\infty) - \log (A_{t_1} - A_\infty))\,(t_2 - t_1) ,$$

using linearity of the time dependence of $\log (A_t - A_\infty)$.

NMR *analysis* All the NMR spectra were measured with a JEOL FX-100 spectrometer (Japan). The ^{13}C NMR spectra were measured at the working frequency of 25·047 MHz, spectral width 6002 Hz (16 kB memory; noise or off-resonace proton decoupling), using the signal ($\delta = 77$) of the solvent C^2HCl_3 ad and internal standard. The ^1H NMR spectra were measured at the frequency of 99·61 MHz, the spectral width 1 000 Hz (8 kB memory; tetramethylsilane as internal standard).

The reaction of 1-(5-nitro-2-furyl)-2-nitroethylene with butylamine was carried out by stepwise dissolving of $2 \cdot 10^{-3}$ mol furylethylene in 2 ml C^2HCl_3 with simultaneous addition of butylamine at −50°C. The mixture was submitted to ^{13}C NMR spectral analysis.

The reaction of 1-(5-nitro-2-furyl)-2-nitroethylene with aniline was carried out in methanol at room temperature, the molar ratio of the two components being 1 : 1. The mixture was analyzed

by TLC (Silufol UV-254; benzene), the starting furylethylene (R_F 0·46) and the product formed (R_F 0·33) being well separated. The fraction containing the reaction product was extracted with acetone, the extract was concentrated in vacuum and analyzed by ^{13}C NMR. The reaction of 1-(5-nitro-2-furyl)-2-nitroethylene with ethanol: $5 . 10^{-4}$ mol furylethylene was dissolved in 100 ml ethanol, whereupon sodium hydroxide was added up to final molar ratio of 1 : 1. After 2 h incubation at room temperature, the mixture was evaporated in vacuum, the residue was dissolved in 3 ml acetone and separated by TLC (Silufol UV-254; benzene-methanol 4 : 1). The addition product (R_F 0·74) was extracted with acetone and submitted to ^1H NMR analysis.

Electrophoresis: After 2 h incubation of 1-(5-nitro-2-furyl)-2-nitroethylene ($4·3 . 10^{-3}$ mol l^{-1} with human serum albumine (1·43 mg protein/ml) in phosphate buffer (pH 8·8), the reaction mixture was submitted to electrophoresis on cellulose acetate at the field voltage of 200 V for 25 min. The separation was carried out with an apparatus of the firm Boskamp (FRG). The spots were made visible by application of a 0·5% solution of Amide Black 10B and evaluated spectrophotometrically at the wavelength of 456 nm using a 1101 M photometer (Eppendorf, FRG) equipped with a compensation recorder 6511 (of the same provenance). The interaction of 1-(5-nitro-2-furyl)-2-nitroethylene with the RNA isolated from the Ehrlich ascitic cells[21] was detected by means of electrophoresis in acrylamide gel according to Loening[22]. The acrylamide and bis-acrylamide concentrations were 4·5 and 0·12% (w/w), respectively. The gels were prepared in 0·04 mol l^{-1} tris(hydroxymethyl)aminomethane–acetate buffer (pH 7·6) containing $2 . 10^{-2}$ mol l^{-1} sodium acetate, $2 . 10^{-3}$ mol l^{-1} sodium ethylenediaminetetraacetate, and 6·0 mol l^{-1} urea. The electrophoresis proceeded at the current intensity of 5 mA/gel at room temperature 75 min. After the electrophoresis, the gels were stained by means of 0·5% Pyronine G solution in a mixture of acetic acid, methanol, and water (1 : 1 : 8) 16 h (Marcinka[23]). The coloured gels were evaluated with a Chromoscan densitometer (Joyce-Loebel; Great Britain).

Inactivation of the Newcastle disease virus: The infection activity of this virus was determined by the plaque assay modified with the method by Dulbecco[24]. The allantoic fluid from chick embryos was clarified by centrifuration at 3 000 rpm for 20 min. Then 0·2 ml virus suspension containing 3·75 m 10^9 infection units per 1 ml was mixed with 0·2 ml (1-(5-nitro-2-furyl)-2-nitroethylene in 0·04 mol l^{-1} phosphate buffer (pH 7·2) with 0·14 mol l^{-1} NaCl so that the resulting furylethylene concentration would be within the required range. The stock solution of furylethylene in dimethyl sulphoxide was applied in such way that the content of this solvent in the reaction mixture might not exceed 0·5% (v/v). After 1 h incubation at room temperature, the mixture was diluted with 0·14 mol l^{-1} NaCl with 1% inactivated calf serum. The mono-layer cultures of chick embryo cells were infected with 0·5 ml of the correspondingly diluted virus and (after 45 min adsorption) with 4 ml solution containing the Earle cultivating medium, 2% of the inactivated calf serum, and 1% agar, pre-heated at 45°C (ref.[25]). After solidification of agar, the plates were submitted to incubation at 37°C in an atmosphere containing 5% CO_2 for 60 h. The cells were stained with a 0·02% solution of Neutral Red in 0·14 mol l^{-1} NaCl with 1% agar. After 5 h incubation at 37°C the number of plaques was estimated.

RESULTS AND DISCUSSION

The mention of possible nucleophilic addition reactions of aliphatic and aromatic amines with compounds containing nitroethylene grouping (β-nitrostyrenes[26], nitroethylene[27]) in organic solvents was also interesting from the point of view of studies of biochemically important reactions of 5-nitro-2-furylethylenes. It already has been shown[10] that compounds of 5-nitro-2-furylethylene type react with thiols

in aqueous medium, the $C_{(1)}$ atom of the exocyclic double bond being attacked by thiolate anion. Therefore, it is supposed that amino and hydroxy groups of both low-molecular and macromolecular compounds could react with 5-nitro-2-furylethylenes in aqueous medium by the same mechanism. As it already was shown in the case of reactions with low-molecular thiols[10,11] the addition-type reactions of 5-nitro-2-furylethylenes can easily be detected by means of spectrophotometry. These derivatives exhibit characteristical electronic absorption maximum in the region of 310–380 nm in aqueous media, and the amines and alcohols studied by us do not absorb in this spectral region. As the reaction products have significantly different absorption electronic spectra from those of the starting 5-nitro-2-furylethylene derivatives, this method can be applied to both demonstration and characterization of reactivity of 5-nitro-2-furylethylene with amines and alcohols. The reactions of 1-(5-nitro-2-furyl)-2-nitroethylene (as a representative of nitrofurylethylenes) with both low-molecular model amines and biologically active amines were carried out in aqueous buffer solutions with an at least 20 fold excess of the reactive ($R—NH_2$) form of the amine with respect to the substrate studied, which ensured the pseudomonomolecular course of the reaction. Butylamine (Fig. 1) as well as the other amines react with

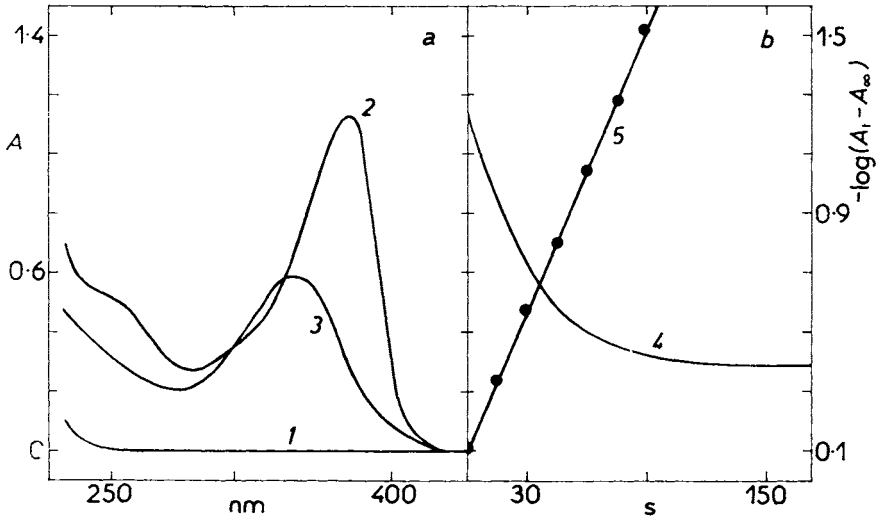


FIG. 1

Electronic absorption spectra *a* butylamine *1*, 1-(5-nitro-2-furyl)-2-nitroethylene 2, their mixture after reaction *3*; *b* reaction kinetics followed at 365 nm (time dependence of A_t *4* and of log $(A_t - A_\infty)$ 5). Initial concentration of butylamine $2 \cdot 10^{-2}$ mol l^{-1}, of 2-furylethylene $5 \cdot 10^{-5}$ mol l^{-1}. The Clark–Lubs buffer pH 7·0, 25°C, 1 cm cell. A_t, A_∞ — absorbance at a time *t* and after the reaction is finished

1-(5-nitro-2-furyl)-2-nitroethylene to give products whose absorption maxima are lower and are shifted towards shorter wavelengths. The kinetic dependences obtained (Fig. 1) could be converted into the second order rate constants[20] of the reactions of 1-(5-nitro-2-furyl)-2-nitroethylene with the said low-molecular amines. Table I shows that these second order rate constants depend on pK_a values of the amino groups. With both the model amines and the biologically active amines the reactivity of the respective amino group increases with increasing pK_a value. The second order rate constants found for the reactions of 1-(5-nitro-2-furyl)-2-nitroethylene with the respective amines are lower by 4–5 orders of magnitude than the reactions with glutathione, coenzyme A, and dihydrolipic acid (as the model thiols). As compared with the other amines, a higher reactivity is only exhibited by butylamine, whose, considerable basicity is accompanied by strong nucleophilicity of its primary aliphatic amino group. The reactivity problems (and their biological importance) of the reactions of furylethylenes with $—NH_2$, —SH, and —OH groups are summarized in detail in ref.[31].

In order to obtain a more detailed description of the reactions of low-molecular amines with 1-(5-nitro-2-furyl)-2-nitroethylenes in aqueous media, we studied products of these reactions by means of NMR spectroscopy. As these reaction products are little soluble in aqueous medium, we carried out their preparation in less polar media. Both spectrophotometrical and chromatographical behaviour of the products thus prepared from 1-(5-nitro-2-furyl)-2-nitroethylene and butylamine (as a repre-

TABLE I

Dissociation constants (pK_a) of the amines and model thiols used and the second order rate constants (k) of reactions of these nucleophilic reagents with 1-(5-nitro-2-furyl)-2-nitroethylene at 25°C

Nucleophile	pK_a (ref.)	k, l mol^{-1} s^{-1}
Butylamine	10·77 (28)	$8{\cdot}7 \cdot 10^{3}$
Glycine	9·80 (19)	$5{\cdot}4 \cdot 10^{0}$
Taurine	9·06 (19)	$6{\cdot}0 \cdot 10^{-1}$
Glucosamine	7·80 (19)	$2{\cdot}7 \cdot 10^{-1}$
Aniline	4·62 (28)	$4{\cdot}4 \cdot 10^{-1}$
Tyramine	10·40 (19)	$1{\cdot}6 \cdot 10^{1}$
Tryptamine	10·20 (19)	$1{\cdot}5 \cdot 10^{1}$
Noradrenaline	9·98 (19)	$6{\cdot}0 \cdot 10^{0}$
Histamine	9·76 (19)	$6{\cdot}9 \cdot 10^{0}$
Dihydrolipoic acid	10·70 (29)	$7{\cdot}8 \cdot 10^{5}$
Coenzyme A	9·60 (29)	$9{\cdot}3 \cdot 10^{5}$
Glutathione	8·63 (30)	$1{\cdot}3 \cdot 10^{5}$

sentative of aliphatic amines) and with aniline (as an aromatic amine) was quite identical with that of the reaction products formed during the corresponding kinetic measurements in aqueous solutions.

The positions and off-resonance multiplicities of the ^{13}C NMR spectral signals of the reaction products of the derivative investigated and the amines indicate that the reaction proceeds as the addition of the amine to the exocyclic double bond. The position of attack by the amine nitrogen atom can be determined from chemical shifts of the sp^3 hybridized carbon atoms: the CH_2 carbon atom shows the chemical shifts 76·1 and 76·9δ (for the products with aniline and butylamine, respectively), the corresponding CH chemical shifts being 50·4 and 54·3δ, resp. Comparison of these chemical shifts with those of the analogous adducts of 5-nitro-2-furylethylenes and thiols[10] indicates unambiguously that both butylamine and aniline are added to the $C_{(1)}$ atom of the exocyclic double bond to give 1-(5-nitro-2-furyl)-1-N-(n-butyl)-amino-2-nitroethane and 1-(5-nitro-2-furyl)-1-N-phenylamino-2-nitroethane, respectively. At the same time it was possible to confirm the presumption[10] that 2-aminoethanethiol reacts with 3-(5-nitro-2-furyl)acrylic acid at the amino group. A similar type of addition reactions can also be expected in the case of interactions of 5-nitro-2-furylethylenes with alcohols. In this case, however, realization of the reactions with simple aliphatic alcohols (methanol, ethanol, *etc.*) in aqueous medium presents considerable difficulties. The dissociation constants of these alcohols have extremely high values[32], and, hence, special reaction conditions are necessary to produce the alkoxide anion concentrations required for the pseudomonomolecular reaction course. One way consists in increasing the alcohol concentration up to the values at which the aqueous character of the medium is suppressed, and the other way is based on extreme increase of basicity of the medium, at which conditions, however, the 5-nitrofurane ring is very unstable. Aromatic hydroxy compounds have usually substantially lower pK_a values, hence the addition reactions of this type are easily realized in aqueous media. We followed spectrophotometrically the reaction of 1-(5-nitro-2-furyl)-2-nitroethylene with phenol (pK_a = 10·0)[19] at analogous reaction conditions as in the case of the above-mentioned reactions of this compound with amines. The second order rate constant found is $k = 1{\cdot}40 \cdot 10^{-1}$ l mol^{-1} s^{-1}. We chose the reaction of 1-(5-nitro-2-furyl)-2-nitroethylene with methanol and ethanol to find whether the nuclephilic addition attack on 5-nitro-2-furylethylenes is also possible with aliphatic alcohols. In the case of this reaction with 95% ethanol in the presence of NaOH we could identify by means of ^1H NMR spectra formation of the addition product, 1-(5-nitro-2-furyl)-1-ethoxy-2-nitroethane. In the 6–8δ region it is possible to identify easily the spectral signals due to two AB proton spin systems of the starting 2-furylethylene and an AB spin system of the protons of furane nucleus in the product. In the region of 4·9–5·5δ there are signals of the ABC spin system of the protons of the ethylene bohd which has reacted. The non-equivalency of the protons of CH_2 group is due to chirality of the neighbouring CH group. Analysis

of this spectral region by means of the LAOCOON program[33] gave the following spectral parameters: $\delta(CH) = 5{\cdot}34$; $\delta(CH_2^a) = 4{\cdot}93$; $(CH_2^b) = 5{\cdot}04$; $^3J(CH—CH_2^a) = 9{\cdot}7$ Hz; $^3J(CH—CH_2^b) = 3{\cdot}4$ Hz; $^2J(CH_2) = -13{\cdot}3$ Hz. The existence of such a spin system confirms unambiguously the addition reaction at the exocyclic double bond. With respect to the fact that the values of chemical shifts of the CH and CH_2 protons are very close, it is obvious that the oxygen atom is bound to the exocyclic $C_{(1)}$ atom. If this were not the case, a large difference (about 4 ppm) between the chemical shifts of the respective protons would be expected. The chirality of the CH group also affects the CH_2 protons of ethoxy group (the respective chemical shifts $\delta = 3{\cdot}60$ and $3{\cdot}64$). This fact also proves the bond of the ethoxy group in the product.

The next part of our work deals with evaluation of the interaction of 1-(5-nitro-2-furyl)-2-nitroethylene with hydroxyl ions. These reactions are interesting with respect to considerable instability of 5-nitro-2-furylethylenes in aqueous medium. In ref.[34] a detailed study is presented of the mechanism of nucleophilic addition of water to β-nitrostyrenes which resemble 5-nitro-2-furylethylenes in having exocyclic β-nitrovinyl grouping. These reactions are initiated by an attack of hydroxyl ion at the $C_{(1)}$ atom of the exocyclic double bond and formation of the addition product which is decomposed to nitromethane and the corresponding benzaldehyde derivative in the subsequent step. Thus the reaction represents a retro-Knoevenagel transformation. An analogous reaction of 1-(5-nitro-2-furyl)-2-nitroethylene is complicated by considerable lability of 5-nitrofurane ring at higher pH values which are necessary because of the low reaction rate. Therefore, we carried out the reaction in 95% methanol with addition of sodium methoxide (the resulting molar ratio of furylethylene to methoxide was 1 : 2), and the final product of the retro-Knoevenagel reaction of 1-(5-nitro-2-furyl)-2-nitroethylene was detected by means of ^{1}H NMR spectra to be 5-nitro-2-furaldehyde. Its spectral parameters were: $\delta(CHO) = 9{\cdot}73$; δ(CH-furane) = 7·46 and 6·86, resp. Therefore, it seems probable that just the formation of 5-nitro-2-furaldehyde could represent one of possible explanations of the high mutagenic activity of some 5-nitrofurylethylene derivatives: the ability of various aldehydes to modify directly nucleic acids has been known for a long time.

Furthermore we tried to investigate the possibility of these reactions (proceeding with low-molecular amines and alcohols) also with their macromolecular analogues. Therefore, we examined the interactions of 1-(5-nitro-2-furyl)-2-nitroethylene with ribonuclease and with human serum albumin. In both the cases the proteins do not contain free SH groups. The reactions were followed spectrophotometrically in phosphate buffer (pH 8·8). The reaction conditions were adjusted in such way that, with the both proteins, the —NH_2 and —OH groups would be present in an at least 20–30 fold excess with respect to concentration of furylethylene (for this ratio we considered ribonuclease to contain eleven —NH_2 groups and thirty —OH groups per one molecule, the corresponding numbers being 63 and 62 for the human serum albumin)[35,36].

From Fig. 2 it is seen that a characteristic change in the region of the absorption maximum of the derivative studied took place after action of both ribonuclease and the human serum albumin. The interaction of the latter with 1-(5-nitro-2-furyl)--2-nitroethylene was proved on the basis of changed mobility of the human serum albumine in electric field. The electrophoresis on cellulose acetate was carried out after incubation of the reaction mixture in phosphate buffer (pH 8·8) at room temperature for 2 h. The electrophoretic mobility of the reaction product was higher by a factor of 1·8 as compared with the original biopolymer. As already mentioned, mutagenity and carcinogenity of many 5-nitro-2-furylethylene derivatives was proved without doubt[13-17]. In this context it would be certainly interesting to demonstrate direct interaction of 1-(5-nitro-2-furyl)-2-nitroethylene with isolated nucleic acids. So far it is also not clear whether these interactions are possible without metabolic activation of this derivative in a biosystem. The interactions were followed spectrophotometrically in a buffer of 0·2 mol l^{-1} tris(hydroxymethyl)aminomethane hydrochloride containing 0·02 mol l^{-1} sodium chloride (pH 7·0). In the region of the absorption maximum of the derivative studied (Fig. 3) a significant change was observed

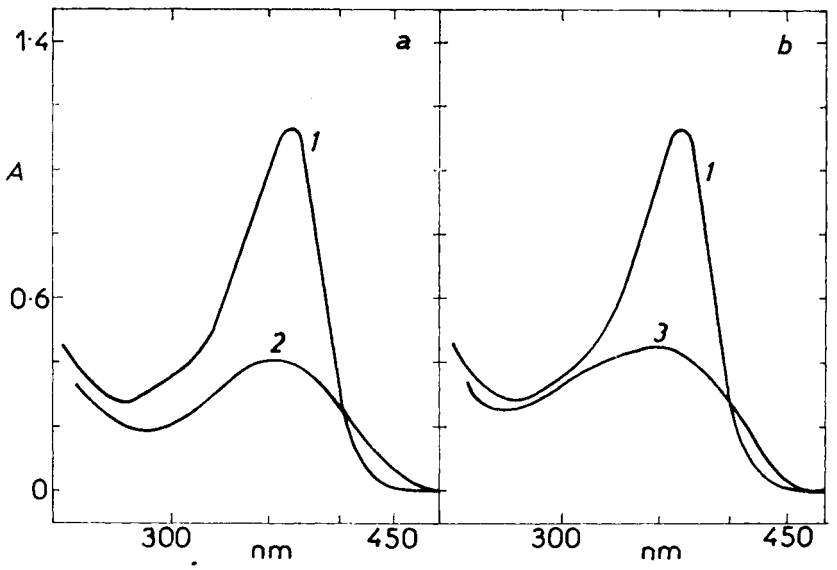


FIG. 2

Electronic absorption spectra of 1-(5-nitro-2-furyl)-2-nitroethylene *1* and its reaction mixtures after reaction with *a* ribonuclease (2·49 mg protein/ml) *2* and *b* with human serum albumin (1·3 mg protein/ml) *3*. Initial concentration of the 2-furylethylene $5 \cdot 10^{-5}$ mol l^{-1}. Phosphate buffer medium (pH 8·6). 1 cm cell

after the reaction with both DNA and RNA. A direct interaction of 1-(5-nitro--2-furyl)-2-nitroethylene with RNA was proved also by means of electrophoresis in polyacrylamide gel. The RNA used was isolated from the Ehrlich ascitic cells[21] and was left to react with the derivative studied at room temperature. Fig. 3 shows the electrophoretic profile of the reaction products. The combination of furylethylene with individual RNA fractions was very well visible during the electrophoresis because of considerably distinct colouration of the added compound and of the products formed. This effect was still deepened by partial degradation of RNA which is increased with time of action of the derivative studied. After 24 h incubation RNA is completely degraded to low-molecular fragments.

The effect of 1-(5-nitro-2-furyl)-2-nitroethylene was also followed on the infectious activity of the virus of the Newcastle disease virus whose genom is macromolecular RNA. Thus we followed the effect of furylethylene on a biologically active form of nucleoprotein in a relatively simple biological system. The suspension of the virus

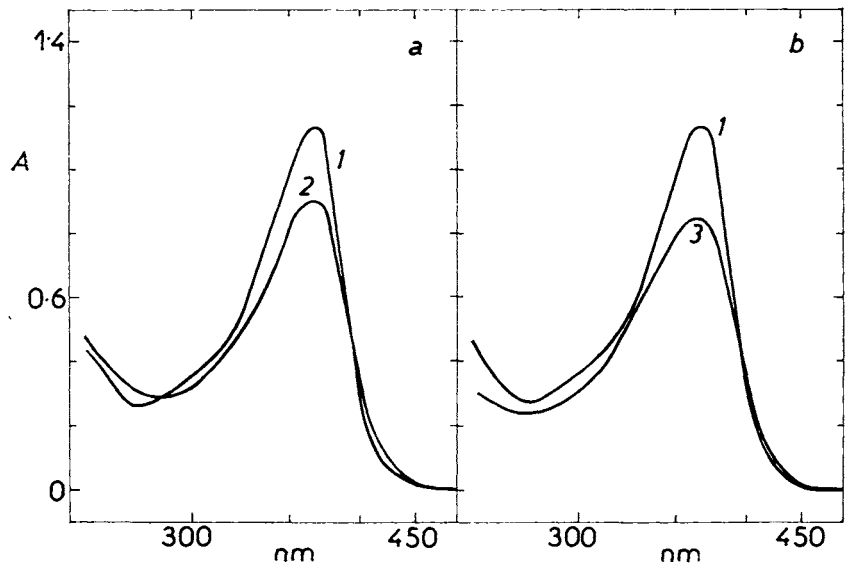


FIG. 3

Spectrometric indication of reaction of 1-(5-nitro-2-furyl)-2-nitroethylene with nucleic acids. Electronic absorption spectra of the 2-furylethylene before *1* and after the action of *a* yeast RNA (0·022 mg/ml) *2*, *b* DNA isolated from cow thymus (0·03 mg/ml) *3*. Initial concentration of the 2-furylethylene was $5 \cdot 10^{-5}$ mol l^{-1}. Reaction medium: aqueous tris(hydroxymethyl)aminomethane hydrochloride (0·02 mol l^{-1}) with NaCl (0·02 mol l^{-1}), pH 7·0, room temperature, 1 cm cell

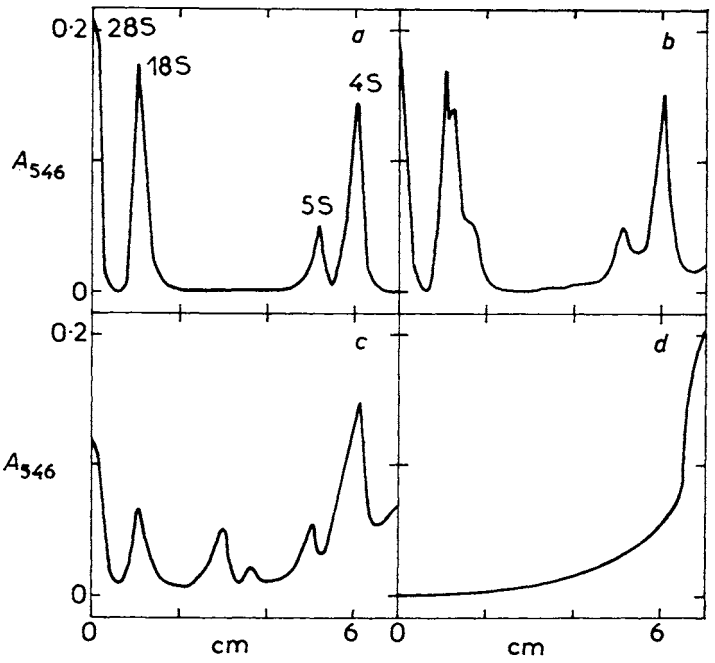
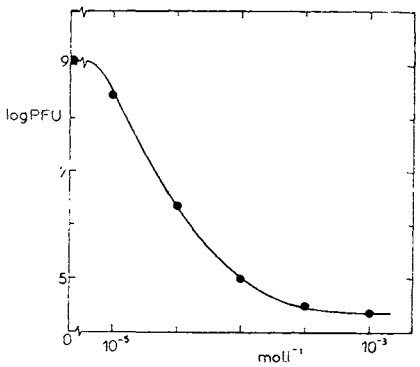


FIG. 4

Electroforetogram of RNA isolated from the Ehrlich ascitic cells separated in polyacrylamide gel before (*a*) and after 2 h (*b*), 4 h (*c*), and 24 h action (*d*) of 1-(5-nitro-2-furyl)-2-nitroethylene. Initial concentration of 2-furylethylene $4 \cdot 10^{-4}$ mol l^{-1}, RNA 1·5 mg/ml. Phosphate buffer medium (0·01 mol l^{-1}; pH 7·6), 25°C. Electrophoresis: current intensity of 5 mA/gel, 75 min, room temperature

FIG. 5

Dependence of logarithm of number of infection units of the Newcastle disease virus (PFU) on the used concentration of 1-(5-nitro-2-furyl)-2-nitroethylene. The nitrofurylethylene was incubated with a suspension of the virus containing $1{\cdot}87 \cdot 10^9$ infection units of the virus, at room temperature for 1 h. The number of infection units of virus was determined by the method of plaque assay



was incubated with the substance studied at room temperature 1 h (the furylethylene concentration $1 . 10^{-3}$ to $1 . 10^{-5}$ mol l^{-1}). The found inactivation effect of 1-(5--nitro-2-furyl)-2-nitroethylene was expressed as the number of the infection units of the virus determined by the plaque assay. Fig. 5 shows that the compound studied exhibits a high inactivation effect on the Newcastle disease virus within the whole concentration range investigated. It is, however, not yet clear whether the inactivation of the virus studied is due to chemical modification of its protein components or that of nucleic acid molecule. Simultaneous modification of the both types of biopolymers cannot be excluded, too, such modification being accompanied by a possibility of formation of cross links between the nucleic acid and proteins[37].

REFERENCES

1. Aiso K., Kanisawa M., Yamaoka H., Tatsumi K., Aikawa N.: J. Food Hyg. Soc. Jpn. *3*, 365 (1962).
2. Cyanamid International: *Payzone Nitrovin, Growth Promoting Agent for Pigs*, p. 16 (1973).
3. Ebringer L., Šubík J., Lahitová N., Trubačík S., Horváthová R., Siekel P., Krajčovič J.: Neoplasma *29*, 675 (1982).
4. Ong T. M., Callen D. F., Huang S. L., Batzinger R. P., Bueding E.: Mutation Res. *48*, 37 (1977).
5. Ong T. M.: Mutation Res. *56*, 13 (1977).
6. Drobnica Ľ., Šturdík E.: Folia Microbiol. (Prague) *25*, 467 (1980).
7. Lu C., Mc Calla D. R.: Can. J. Microbiol. *24*, 650 (1978).
8. Drobnica Ľ., Šturdík E., Kováč J., Vegh D.: Neoplasma *28*, 281 (1981).
9. Šturdík E., Drobnica Ľ., Baláž Š., Marko V.: Biochem. Pharmacol. *28*, 2525 (1979).
10. Šturdík E., Liptaj T., Baláž Š., Drobnica Ľ.: This Journal *47*, 1523 (1982).
11. Baláž Š., Šturdík E., Drobnica Ľ.: This Journal *47*, 1659 (1982).
12. Šturdík E., Drobnica Ľ., Baláž Š.: This Journal *48*, 327 (1983); *48*, 336 (1983).
13. Yahagi T., Matsushima T., Nagao M., Seino Y., Sugimura T., Bryan G. T.: Mutat. Res. *40*, 9 (1979).
14. Mc Calla D. R. in the book: *Mechanism of Action of Antibacterial Agents* (F. E. Haku, Ed.), Vol. 1, p. 176. Springer, Berlin 1979.
15. Mc Calla D. R.: Environ. Mutagen. *5*, 745 (1983).
16. Yahagi T., Nagao M., Hara K., Matsushima T., Sugimura T., Bryan G. T.: Cancer Res. *34*, 2266 (1974).
17. Bryan G. T. (Ed.): *Carcinogenesis*, Vol. 4, *Nitrofurans*, Raven Press, New York 1978.
18. Nazarov Z. N.: Zh. Obshch. Khim. *24*, 575 (1954).
19. Dawson R. M. C., Elliot D. C., Elliot W. H., Jones K. M.: *Data for Biochemical Research*. Clarendon Press, Oxford 1969.
20. Frost A. A., Pearson R. G.: *Kinetics and Mechanism*, 2nd Ed., p. 49. Wiley-Interscience, New York 1961.
21. Rosenbergová M., Pristašová S.: Acta Virol. *16*, 1 (1972).
22. Loening U. E.: Biochem. J. *102*, 251 (1967).
23. Marcinka K.: Annal. Biochem. *50*, 304 (1972).
24. Dulbecco R.: Proc. Natl. Acad. Sci. *38*, 747 (1952).
25. Leško J., Veber P., Hána L.: *Práce s tkanivovými kultúrami*. Věda, Published by SAV, Bratislava 1975.

26. Lough C. E., Currie D. J., Can. J. Chem. *44*, 1563 (1966).
27. Ranganathan D., Ranganathan S., Bamezai S.: Tetrahedron Lett. *23*, 2789 (1982).
28. Gordon J. A., Ford A. R.: *The Chemist's Companion.* Wiley-Interscience, New York 1972.
29. Podhradský D., Drobnica Ľ., Kristián P.: Experientia *35*, 154 (1979).
30. Friedman M., Cavins J. F., Wall J. S.: J. Amer. Chem. Soc. *87*, 3672 (1965).
31. Baláž Š., Šturdík E., Rosenberg M., Végh D.: This Journal, in press.
32. Houben-Weyl: *Methoden der Organischen Chemie*, Band VI/1a, Teil 1: *Alkohole* I, Thieme, Stuttgart 1979.
33. De Los F. de Tar (Ed.) *Computer Programs for Chemistry*, Vol. 1, p. 10. Benjamin, New York 1968.
34. Crowell T. I.: J. Org. Chem. *48*, 3294 (1983).
35. Hias C. H. W., Moore S., Stenion W. H.: J. Biol. Chem. *219*, 623 (1956).
36. Spahr P. F., Ed. Sall J. T.: J. Biol. Chem. *239*, 850 (1964).
37. Angelis K.: Acta Hyg. Epid. (Supplement 8), p. 56 (1980).

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