

**REACTION OF ISOTHIOCYANATES WITH AMINO ACIDS,
PEPTIDES AND PROTEINS. III.***
**KINETICS AND MECHANISM OF THE REACTION OF AROMATIC
ISOTHIOCYANATES WITH THIOGLYCOLIC ACID**

L. DROBNICA and J. AUGUSTÍN

*Institute of Technical Microbiology and Biochemistry, Chemical Faculty,
Slovak Technical University, Bratislava*

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Isothiocyanates react with thioglycolate by the ionized sulphhydryl group. The dependence of the reaction rate on pH has the shape of a dissociation curve. The reaction products under the investigated conditions are carboxymethyl N-aryldithiocarbamates which are cyclized in acidic medium.

The rate constants k of the reaction of arylisothiocyanates with thioglycolate are higher by two orders of magnitude in average than the rate constants of the reaction of the arylisothiocyanates with glycine or OH^- ions (the reactivity was investigated under identical conditions). The relative reaction rates of the investigated arylisothiocyanates with thioglycolate give a linear dependence when plotted against the same values for the reaction with OH^- ions or with glycine. The values of the Arrhenius activation energy and of the logarithm of the frequency factor fulfill Leffler's isokinetic relationship.

Our experiments, directed towards studies of the reactions of isothiocyanates with the sulphhydryl groups of amino acids, peptides and proteins, were first oriented towards investigation of the kinetics and mechanisms of the reaction of several arylisothiocyanates with thioglycolic acid, cysteine and reduced glutathione. The importance of detailed studies of the reactions of isothiocyanates with thiol compounds is indicated by our preceding work on the role of the isothiocyanates in the metabolism of yeast¹, Ehrlich ascites tumor cells or HeLa cells^{1,3}, which consists in the inhibition of glycerol aldehyde-3-phosphate dehydrogenase. This as well as some other enzymes containing sulphhydryl groups are sensitive to isothiocyanates also in isolated form. Inactivation can be prevented by adding thiol compounds (*e.g.* thioglycolic acid). In this paper the results of our studies of the reactions of arylisothiocyanates with the SH-group of thioglycolic acid are summarized. The papers^{4,5} are concerned with the reaction of this compound with the NH_2 group of amino acids.

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Experimental

Chemicals and Apparatus

The kinetics of the reactions of isothiocyanates with thioglycolic acid were investigated with the same derivatives as described previously⁴. The buffers were prepared with p.a. chemicals from Lachema. The pH value was adjusted by an electronic pH-meter using a glass and calomel electrode. The isothiocyanates were usually dissolved in methanol. The experiments were always carried out with fresh solutions. The required sodium thioglycolate (Lachema) concentration in aqueous solutions (boiled redist. water) was adjusted after iodometric determination of the concentration of the reduced form. Inert atmosphere in the reaction of isothiocyanates with thiol compounds was achieved by bubbling the reaction mixture with oxygen-free nitrogen. The spectrophotometric measurements were carried out with the Zeiss VSU-1 instrument in an air conditioned room.

The course of the reaction of aromatic isothiocyanates with thioglycolate was followed spectrophotometrically in the range of the adsorption maxima in the ultraviolet range of the isothiocyanate⁴ or of the reaction products. The reaction medium in the determination of the apparent rate constants (k') was the citrate-phosphate buffer of McIlvain of pH 7.5 (0.1M citric acid + 0.2M Na_2HPO_4). The initial isothiocyanate concentration was usually $3.13 \cdot 10^{-5}\text{M}$. The concentration of thioglycolate in the form of $(^-)\text{S}_2\text{CH}_2\text{COO}(-)$, maintained with the aid of the buffer, was $1.25 \cdot 10^{-3}\text{M}$ ($\text{p}K_{\text{SH}} = 10.0$)⁶. The volume of the reaction mixture was 12 ml, quartz vessels with an internal diameter of 30 mm (11.7 ml of buffer, 0.15 ml of $2.5 \cdot 10^{-3}\text{M}$ isothiocyanate in methanol and 0.15 ml of 0.1M sodium thioglycolate in water) were used. The measurements were carried out at a temperature of $25 \pm 0.2^\circ\text{C}$. With selected isothiocyanates the temperature dependence was investigated at four different temperatures in the range from 20 to 40°C .

In the experiments where the effect of pH of the reaction medium on the reaction rate was followed, Clark-Lubbs phosphate buffer was used. The reaction mixture contained 39.4 ml of buffer of the corresponding pH, 0.5 ml of the aqueous thioglycolate (0.1M) solution and 0.125 ml of a phenylisothiocyanate solution (0.01M) in methanol or other isothiocyanates. The samples were kept in the ultrathermostat at 20°C and the extinction of the reaction mixture was determined at 300 nm (cuvette of internal diameter 20 mm) in suitable intervals (selected on the basis of preliminary measurements).

Under the conditions of the investigations of the reaction of isothiocyanates with thioglycolate the reaction proceeded according to the equation for a first order reaction. The reaction rate significantly depends on the hydrogen ion concentration. In a similar way as in the case of the reaction with amino acids⁵, the reaction of thioglycolate with isothiocyanates is also preceded by a dissociation reaction (thioglycolate reacts with the ionized sulphhydryl group). Since the reactivity of the isothiocyanates with thioglycolate is very much higher than that with OH^- ions⁴, side reactions of the isothiocyanates with OH^- ions were negligible under the given conditions.

The values of the apparent rate constants k' of the reaction of the isothiocyanates with thioglycolate were obtained from the slope of the linear dependence from $\log(E_0 - E_\infty)$ plotted against time. The values of the Arrhenius activation energy (E) and of the logarithm of the frequency factor ($\log A$) were calculated by a similar method as in the case of the studies of the reactions with amino acids.

Synthesis of the Reaction Products of Isothiocyanates with Thioglycolate

Besides the main products of the reaction of the investigated isothiocyanates with thioglycolate (i.e. of the carboxymethyl N-aryldithiocarbamates) in the reaction mixtures of neutral or weakly acidic pH (in studies of the dependence of k on pH) a certain fraction of these products could be cyclized to the corresponding N-arylrhodanine. In order to identify the reaction products of the

isothiocyanates with thioglycolate N-phenylrhodanine (m.p. 192°C) was prepared by the described method⁷ and according to the procedure reported by Elmore and co-workers, for the preparation of carboxymethyl-N-benzoyldithiocarbamate⁸. The carboxymethyl-N-phenyldithiocarbamate was prepared which was identified as the N-phenylrhodanine after cyclization in glacial acetic acid saturated with hydrogen chloride and evaporation of the solvent (m.p. 192°C).

Results and Discussion

Comparison of the UV absorption curves of the reaction mixtures after carrying out the reaction of the phenylisothiocyanate with thioglycolate (McIlvain buffer of pH 7.5 and temperatures up to 40°C or phosphate buffer according to Clark-Lubbs at a temperature of 20°C at various pH in the range of 5.6–8.0 with addition of the reactants) and of the UV absorption curves of N-phenylrhodanine or carboxymethyl-N-phenylrhodanic acid or carboxymethyl-N-phenyldithiocarbamate showed that

Table I

Apparent and Effective Rate Constants of the Reaction of Phenylisothiocyanates with Thioglycolate in Clark-Lubbs Phosphate Buffer at Various pH
Initial thioglycolate concentration $1.25 \cdot 10^{-3} \text{ M}$ ($pK_{SH} 10.00$ at 20°C).

pH	Concentration of the Ionized Form $\text{mol} \cdot \text{l}^{-1} \cdot 10^{-7}$	$10^{-2} \cdot k'$ min^{-1}	$10^4 \cdot k$ $\text{l} \cdot \text{mol}^{-1} \cdot \text{min}^{-1}$
6.5	3.95	2.4	6.2
7.0	12.5	5.9	4.7
7.6	49.6	22.3	4.5
8.0	124	62.5	5.1

Table II

Parameters of the Arrhenius Reaction Equation of the Arylisothiocyanates $\text{R}-\text{C}_6\text{H}_4-\text{NCS}$ with Thioglycolate in Citrate-Phosphate Buffer (McIlvain, pH 7)
The dependence of reactivity on temperature was investigated in the range 20–40°C.

R	E $\text{kcal} \cdot \text{mol}^{-1}$	$\log A$ $\text{l} \cdot \text{mol}^{-1} \cdot \text{min}^{-1}$
4-Cl	16.4 ± 0.7	6.6 ± 0.3
4-J	27.1 ± 0.9	14.6 ± 0.7
4- CH_2COOH	14.3 ± 0.6	5.1 ± 0.2
4- CH_3CO	8.3 ± 0.4	0.15 ± 0.01
4-Br	9.4 ± 0.4	1.4 ± 0.06
H	12.1 ± 1.0	3.5 ± 0.2

Table III

Rate Constants and Relative Reaction Rates of Substituted Phenylisothiocyanates $R-C_6H_4-N=C=S$ with Thioglycolate (McIlvain Buffer of pH 7.5; 25°C) and with Glycine (Borate Buffer pH 9.8; 25°C)

R	Reaction with Thioglycolate ^a		Reaction with Glycine ^{b,c}	
	$k \cdot 10^4$ l. mol. min ⁻¹	$\Delta \log k$	k l. mol. min ⁻¹	$\Delta \log k$
4-H	6.3 ± 0.3	0.0000	14.6 ± 0.6	0.0000
4-Cl	18.6 ± 0.8	0.4702	22.2 ± 2.0	0.1820
4-J	15.7 ± 0.7	0.3966	24.8 ± 3.2	0.2301
4-Br	11.6 ± 0.4	0.2652	21.1 ± 1.3	0.1599
3-Br	19.5 ± 0.8	0.4907	29.8 ± 2.3	0.3098
4-HO	3.1 ± 0.1	-0.3079	8.5 ± 1.2	-0.2350
4-NO ₂	~56	0.9489	~163	1.0500
4-COOH	12.9 ± 0.5	0.3113	16.8 ± 0.9	0.0609
3-COOH	4.1 ± 0.2	-0.1865	10.8 ± 1.2	-0.1310
4-CH ₃ CO	21.0 ± 0.8	0.5229	42.2 ± 2.4	0.4609
4-CH ₂ COOH	1.9 ± 0.1	-0.5205	16.8 ± 0.9	0.0609
4-N(CH ₃) ₂	1.4 ± 0.1	-0.6534	5.9 ± 0.6	-0.3935
1-Naphthylisothiocyanate	4.1 ± 0.2	-0.1865	9.2 ± 1.2	-0.2006
4-Brombenzylisothiocyanate	~ 6.3	0.0000	5.8 ± 0.9	-0.4010

^a Initial concentration of isothiocyanates $3 \cdot 13 \cdot 10^{-5} M$ and of a thioglycolate; ^b initial concentration of isothiocyanates $5 \cdot 10^{-5} M$ and of glycine in base form $2.5 \cdot 10^{-3} M$; ^c from^{4,5}.

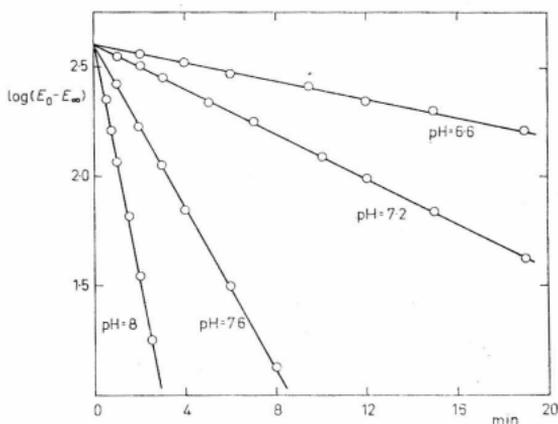


Fig. 1

Course of the Reaction of Phenylisothiocyanate with Thioglycolate in Media of Various pH (Clark-Lubbs phosphate buffer), investigated spectrophotometrically at $\lambda = 300 \text{ nm}$ and 20°C.

the uncyclized form is the only reaction product under the given conditions. Cyclization occurs at temperatures above 40°C and below pH 7. The cyclization rate depends on the pH of the medium.

The apparent rate constant k' of the reaction of the phenylisothiocyanate with thioglycolate, determined at various pH of the reaction mixture (results in Table I, and Fig. 1) indicate that the dependence of the reaction rate on pH of the reaction mixtures has the form of a dissociation curve. The reacting form is therefore the thioglycolate anion with the ionized sulphhydryl group whose concentration is given by the dissociation equilibrium (equation A). The concentration of the ionized form and therefore also the value of k increase with increasing pH value. The product *I* of the addition of the dianion with isothiocyanate in acidic medium cyclizes under release of water to the corresponding N-arylrhodanine *II* which decyclizes in alkaline medium (equation B).

The values of the rate constants of the reaction of 13 arylisothiocyanates and of *p*-bromobenzylisothiocyanate with thioglycolate and with glycine are given in Table II. The relative rates were calculated using unsubstituted phenylisothiocyanate as the reference compound. Determination of the rate constants of six suitably chosen arylisothiocyanates with thioglycolate at various temperatures, calculation of the parameters of the Arrhenius equation (Table III) and plotting of the dependence of the activation energy on the logarithm of the frequency factor (Fig. 2) showed that the investigated derivatives fulfil Leffler's isokinetic relationship also with regard to the reaction with thioglycolate.

Already comparison of the values of the relative rates of the reaction of isothiocyanates with thioglycolate and glycine (Table II) shows the similarity of the sequence

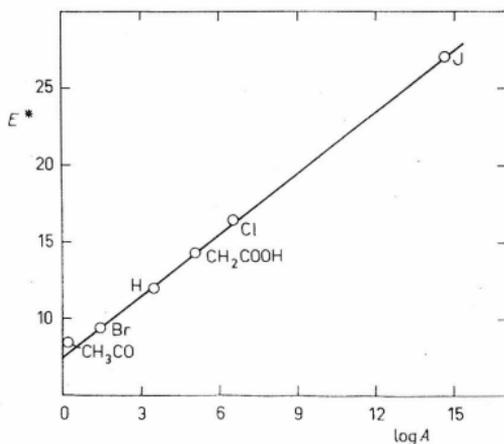


Fig. 2

Dependence of the Activation Energy of the Reaction of Arylisothiocyanates with Thioglycolate on the Logarithm of the Frequency Factor (see Table II)

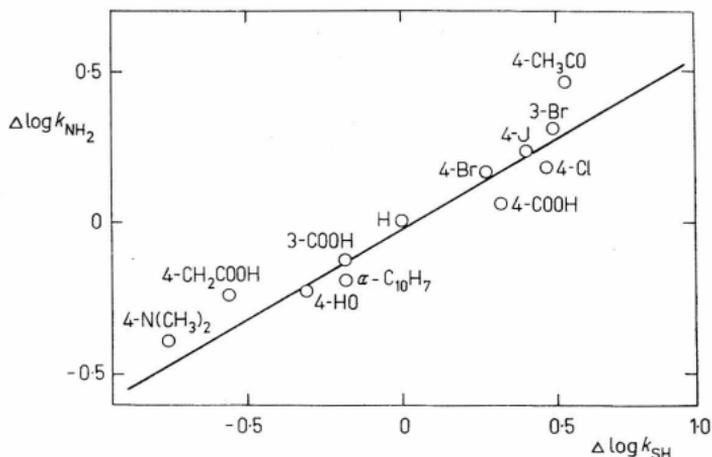


Fig. 3

Dependence of the Relative Rates of the Reaction of Arylisothiocyanates with Thioglycolate (25°C) on the same Terms for the Reaction of Arylisothiocyanates with Glycine $\text{tg } \alpha = 0.587 \pm 0.004$. Designation of the derivatives in Table III. Correlation coefficient $r = 0.956$.

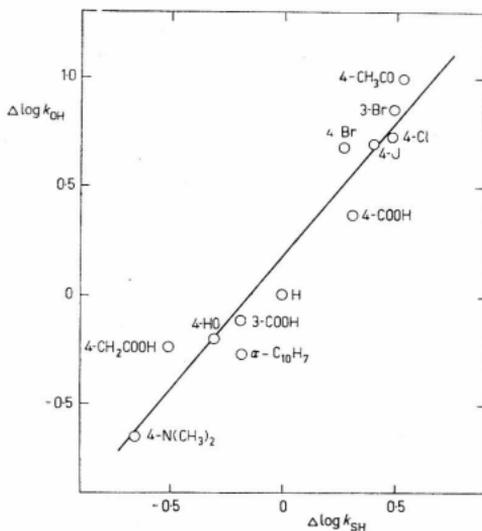


Fig. 4

Dependence of the Relative Rates of the Reaction of Arylisothiocyanates with Thioglycolate (25°C) on the same Terms for the Reaction of Arylisothiocyanates with OH^- Ions (30°C)⁹ $\text{tg } \alpha = 1.240 \pm 0.014$. Correlation coefficient $r = 0.964$.

of the isothiocyanates arranged according to their reactivity to thioglycolate and to glycine. This fact becomes still more apparent from the dependence plotted in Fig. 3. A similar dependence of the values of $\Delta \log k_{SH}$ and $\Delta \log k_{OH^-}$ is shown in Fig. 4.

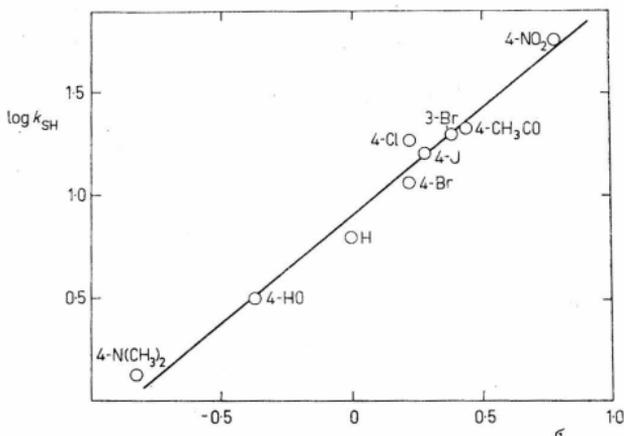


Fig. 5

Relationship between the Logarithms of the Rate Constants of Substituted Phenylisothiocyanates with Thioglycolate (25°C) and Hammett's Constants σ
 Constants σ according to paper¹⁰; $\rho = 1.006 \pm 0.001$; correlation coefficient $r = 0.988$.

It follows that there exists a similar relationship between structure and reactivity of the investigated isothiocyanates to thioglycolate as observed in studies of the reactivity to amino acids^{4,5} and OH⁻ ions⁹. It is therefore possible to correlate the rate constants of the reaction of the isothiocyanate with thioglycolate with the aid of Hammett's equation (Fig. 5). With decreasing electron density on the carbon atom of the functional group NCS the reactivity of the isothiocyanates increases as well. However, it is important to note that the rate constants of the addition reaction of the investigated isothiocyanates with thioglycolate are higher by two orders of magnitude in average than the values of k for the reaction with glycine, other amino acids^{4,5} or with OH⁻ ions⁹. In all three cases addition reactions are concerned which have to be taken into consideration in the elucidation of the reactions of isothiocyanates with cell components. The comparatively high reactivity of isothiocyanates to the thioglycolate anion observed in this paper can serve as further proof for the correctness of the conclusions drawn from the experimental results on the macromolecular and cellular level^{1,3}.

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Резюме

Л. Дробница и И. Аугустин: *Реакции изотиоцианатов с аминокислотами и белками. III. Кинетика и механизм взаимодействия ароматических изотиоцианатов с тиогликолевой кислотой.* Изотиоцианаты взаимодействуют с тиогликолятом посредством ионизованной сульфгидрильной группы. Зависимость скорости реакции от pH имеет форму кривой диссоциации. В данных условиях продуктами реакции являются карбоксиметил-N-арилдитиокарбаматы, в кислой среде подвергающиеся циклизации.

Константы скорости k реакции изотиоцианатов с тиогликолятом примерно на два порядка выше констант скорости реакции изотиоцианатов с глицином или ионами OH^- (реакционная способность исследовалась в тех же условиях). Относительные скорости исследовавшихся арилзотиоцианатов с тиогликолятом, отложенные против тех же значений для реакции с ионами OH^- или глицином, дают линейную зависимость. Значения энергии активации Аррениуса и логарифма частотного фактора удовлетворяют изокинетическому соотношению Леффлера.