

KINETIC ANALYSIS OF COMPETITIVE REACTIONS OF ISOTHIOCYANATES WITH HYDROXYL AND ALKOXIDE IONS

Jozef AUGUSTÍN and Štefan BALÁŽ

*Department of Biochemical Technology,
Slovak Institute of Technology, 812 37 Bratislava*

Received December 9th, 1985

The reaction of isothiocyanates with hydroxyl and alkoxide ions has been followed spectrophotometrically in alkaline aqueous-alcoholic solutions. Both the nucleophiles are added to the carbon atom of isothiocyanate group in a competition reaction. The effects of concentration of the nucleophiles, temperature, and acid-base properties of the alcoholic group have been followed on the reaction course. The possibility of analogous reactions in real biological systems is discussed.

Isothiocyanates are both natural and synthetical compounds with a broad spectrum of antimicrobial effects¹. Among their biochemically important reactions thoroughly investigated are the reactions with amino group (amines, aminoacids^{2,3}), with thiol group (thioglycollic acid⁴, cysteine and its esters, glutathione, coenzyme A, dihydro-lipoic acid⁵), and hydroxyl ions⁶. The knowledge about the reactions of isothiocyanates with alcoholic OH group (which plays an important role in biochemical catalysis⁷) can be denoted as scanty, as it only follows from the experience of preparative organic chemistry⁸⁻¹⁰.

The aim of this work was to investigate the reaction possibilities of isothiocyanates with aliphatic OH group. Low-molecular alcohols have been used as model compounds for this purpose.

EXPERIMENTAL

The commercially available R¹ isothiocyanates were purified by vacuum distillation (R¹ = - phenyl, benzyl) or recrystallization from dioxane (R¹ = 4-bromophenyl). The alcohols used were refluxed with CaO and distilled. The dioxane used for preparation of the solutions was distilled with sodium metal. The alkali solutions were prepared from *p.a.* NaOH and water, those with low water content by dissolving sodium metal in the respective alcohol and addition of the required amount of water.

The monothiourethanes *I* were prepared in the following way: 20 ml respective alcohol was treated with 1 ml aqueous NaOH (6 mol dm⁻³), 0.05 mol isothiocyanate *III*, and the mixture was stirred at 40–50°C for 1 h. The product was precipitated by addition of a fivefold volume of water and acidification to pH 1. The UV absorption spectra and the reaction kinetics were measured with a Specord UV VIS spectrophotometer, the melting temperatures were determined with a Boetius apparatus.

The reaction kinetics were followed spectrophotometrically in two ways: by the decrease of the isothiocyanate *III* and by the increase of the monothiourethane *I* after decomposition of the monothiocarbamate *II* by acidification of the reaction mixture. The reaction mixture containing the required alcohol and NaOH concentrations and placed in a thermostat was treated with a solution of isothiocyanate in dioxane to make the resulting isothiocyanate concentration $5 \cdot 10^{-5} - 1 \cdot 10^{-3} \text{ mol dm}^{-3}$ and that of dioxane 1 vol. %. At suitable time intervals the absorbance was measured at the absorption maximum of isothiocyanate (in the first case) or of the monothiourethane formed (in the second case) after taking the sample into a HCl solution (0.1 mol dm^{-3}). If in the Scheme 1 all the reactants are present in sufficient excess with respect to compounds *I*, *II*, *III*, then the scheme can be simplified to a branched reaction in which compound *III* is transformed into *I* and *II* with the rate constants k_I and k_{II} , respectively. Then the concentration-time dependence of compounds *III* and *I* reads as follows:

$$c_{III}(t) = c_{III}(0) \exp(-(k'_I + k'_{II})t) \quad (1)$$

$$c_I(t) = c_{III}(0) k'_I (1 - \exp(-(k'_I + k'_{II})t)) / (k'_I + k'_{II}), \quad (2)$$

where $k'_I = k_I c_{OR}$, $k'_{II} = k_{II} c_{OH}$, and c is the concentration of the component given in the subscript. The concentrations of alkoxide and hydroxyl ions do not practically change, as they are sufficiently greater than those of compounds *I*, *II*, and *III*.

The overall rate constant observed, $k' = k'_I + k'_{II}$ was determined as the slope of linear dependence $\ln |A - A_e|$ vs time (A is the absorbance and the subscript e refers to the equilibrium state), the individual observed rate constants were calculated from the k' value by application of Eq. (3)

$$c_I/c_{II} = k'_I/k'_{II} \quad (3)$$

the concentrations being determined spectrophotometrically after the reaction was finished (wherefor the fact was made use of that the monothiocarbamate *II* is decomposed in acid medium). The rate constants were obtained as the ratio of the observed rate constant and the concentration of the respective nucleophile calculated with application of the equilibrium constant K (Scheme 1) under the presumption that the equilibrium concentrations of alcohol and water are the same as the initial ones. The K values for various methanol concentrations were determined by graphical interpolation of known data¹¹. The temperature dependence of K was calculated from the van t'Hoff equation for $\Delta H = 14.64 \text{ kJ mol}^{-1}$ (ref.¹²).

RESULTS

The Kinetic Characterization

In alkaline medium isothiocyanates are converted into monothiocarbamates⁶. In excess alcohol, isothiocyanates give monothiourethanes (Table I). The same products were also observed in the presence of other primary and secondary aliphatic alcohols. However, only the respective monothiocarbamates were formed from tertiary alcohols in aqueous alkaline solutions. The UV absorption spectra of phenyl isothiocyanate and those of its reaction products in alkaline aqueous or methanolic media are considerably different (Fig. 1). Hence spectrophotometry represents

TABLE I

The characterization of the monothiourethanes $R^1-NH-CS-OR^2$ prepared: elemental analyses (calculated/found), melting points (published^{10,14}/found) and the wavelengths of absorption maxima (0.1 mol dm^{-3} HCl)

R^1	R^2	Formula (mol. mass)	Elemental analysis, %				M.p. °C	λ_{max} nm
			C	H	N	S		
C_6H_5	CH_3	C_8H_9NOS (167.2)	57.46	5.42	8.38	19.17	92–93	270
			57.40	5.39	8.35	19.15	92	
C_6H_5	C_2H_5	$C_9H_{11}NOS$ (181.2)	59.66	6.12	7.73	17.69	67–68	270
			59.58	6.15	7.64	17.76	66	
C_6H_5	C_3H_7	$C_{10}H_{13}NOS$ (195.3)	61.50	6.71	7.17	16.42	43–44	270
			61.57	6.65	7.22	16.32	43	
4-Br- C_6H_4	C_2H_5	$C_9H_{10}BrNOS$ (260.1)	41.56	3.87	5.38	12.33	104–105	275
			41.61	3.85	5.21	12.24	103	
4-Br- $C_6H_4-CH_2$	C_2H_5	$C_{10}H_{12}BrNOS$ (274.2)	43.80	4.41	5.11	11.71	—	243
			43.89	4.45	5.02	11.64	48	
4- $C_2H_5-O-C_6H_4$	C_2H_5	$C_{11}H_{15}NO_2S$ (225.3)	58.64	6.71	6.22	14.25	95	275
			58.61	6.70	6.27	14.31	94	

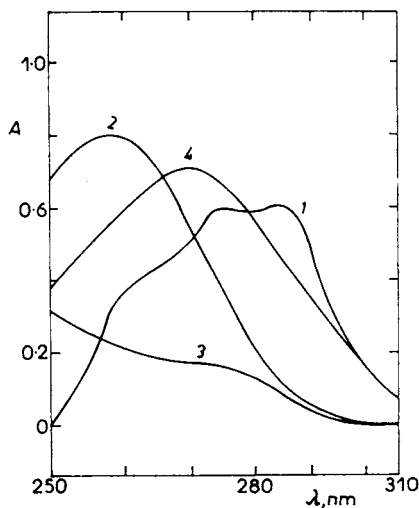
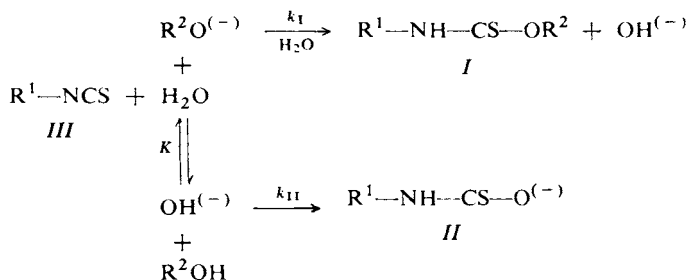


FIG. 1

The UV absorption spectra of phenyl isothiocyanate in methanol (1) and of its reaction products with hydroxyl and alkoxide ions: phenyl monothiocarbamate in $0.1 \text{ mol} \cdot \text{dm}^{-3}$ NaOH (2), phenyl monothiourethane in alkaline (3, 0.1 mol dm^{-3} NaOH) and acidic medium (4, 0.1 mol dm^{-3} HCl) in 1 cm cell

a suitable method to follow the reaction course of isothiocyanates in alkaline aqueous-alcoholic solutions. Such a monitoring of the reaction course is presented in Fig. 2. The yield of monothiourethane increases with increasing alcohol concentration in the reaction mixture. As an addition of alcohol to alkaline solution of monothiocarbamate does not cause any formation of the respective monothiourethane, the reaction is a branched one and not a consecutive one. The fact that in the pure solvents (alcohols, water) the reaction rate is negligibly low indicates that the nucleophiles enter the addition in the much more reactive ionized form, which agrees with literature data¹³⁻¹⁵. The reaction course can be represented by Scheme 1.



SCHEME 1

The reaction kinetics can be followed directly in the alkaline medium or after acidification of the reaction mixture which results in decomposition of monothiocarbamate *II* (ref.¹⁵):

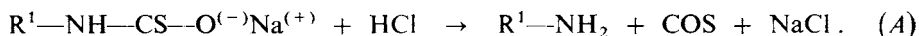


Fig. 3 presents the second way of the monitoring of kinetics. In both the cases we obtain, in this way, the overall observed reaction rate constant which is the sum of the observed rate constants of the two steps. These constants can be separated with the use of Eq. (3), if the concentration ratio of the products *I* and *II* is known.

From Fig. 4 it is seen that the $c_{\text{I}}/c_{\text{II}}$ ratio shows linear dependence on the alcohol to water concentration ratio. This fact agrees with the kinetic description used, as it follows from Eq. (4) which results from Eq. (3).

$$c_{\text{I}}/c_{\text{II}} = (k_{\text{I}}K/k_{\text{II}}) X_{\text{ROH}}/X_{\text{H}_2\text{O}}, \quad (4)$$

where K means the equilibrium constant of the reaction of alcohol with hydroxyl ions (Scheme 1), $K = [\text{RO}^{(-)}] X_{\text{H}_2\text{O}}/[\text{OH}^{(-)}] X_{\text{ROH}}$. The expression $k_{\text{I}}K/k_{\text{II}}$ is practically independent of the composition of the reaction mixture (according to Table II it assumes the values from 16.4 to 18.2 except for $X_{\text{MeOH}} = 0.894$ and 0.660).

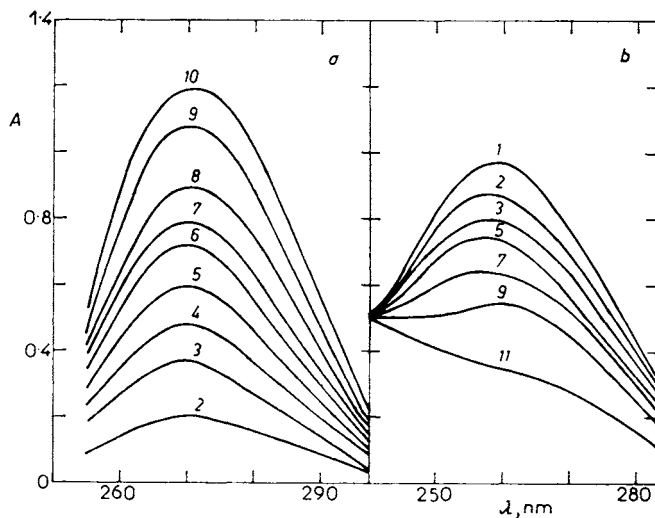


FIG. 2

The UV absorption spectra of the reaction mixture of phenyl isothiocyanate in aqueous-alcoholic solution of NaOH (0.1 mol dm^{-3}) after finishing the reaction: a) after acidification of the reaction mixture with HCl to pH 1 (the spectrum of monothiourethane); b) in the original alkaline medium (the spectrum of both monothiocarbamate and monothiourethane). The methanol concentrations (mol dm^{-3}): 0.025 (1), 0.05 (2), 1.0 (3), 1.5 (4), 2.0 (5), 3.0 (6), 4.0 (7), 5.0 (8), 7.5 (9), 8.7 (10), and 10.0 (11). In a 1 cm cell

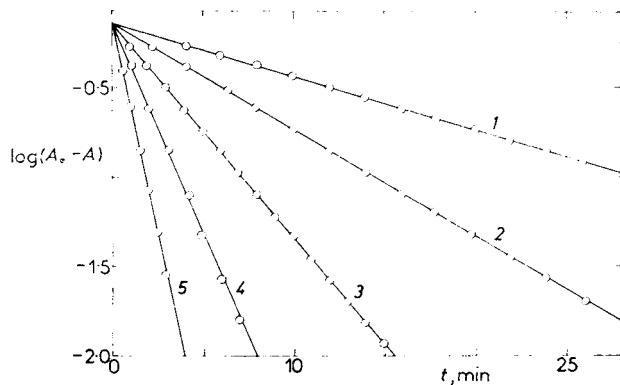


FIG. 3

The kinetics of the reaction of benzyl isothiocyanate in an aqueous-methanolic solution of NaOH at 25°C . The rate of formation of the monothiourethane $\text{C}_6\text{H}_5\text{—NH—CS—O—CH}_3$ was followed by the absorbance changes at 243 nm. The samples were taken into ninefold excess (v/v) of 0.1 mol dm^{-3} HCl. The concentrations of methanol 5 mol dm^{-3} , of NaOH: 5 (1), 10 (2), 20 (3), 40 (4), and 80 (5) mmol dm^{-3}

TABLE II

The rate constants of the reaction of phenyl isothiocyanate with methoxide and hydroxyl ions at the conditions of a branched reaction of the first order. Total concentration of the bases 0.1 mol dm^{-3} ; X is the mol fraction of methanol; K is the equilibrium constant (see Scheme 1) interpolated from known¹¹ data; c_{OR} and c_{OH} are the concentrations of methoxide and hydroxyl ions in mmol dm^{-3} calculated from the definition of the K constant with the presumption that the equilibrium concentrations of methanol and water are the same as the initial ones; k' is the total rate constant observed; k'_{OR} and k'_{OH} are the individual rate constants observed, which were calculated from the k' value with application of Eq. (3), all of them are in s^{-1} ; k_{OR} and k_{OH} are the respective rate constants (both in $\text{mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$) calculated as $k_{\text{OR}} = k'_{\text{OR}}/c_{\text{OR}}$ and $k_{\text{OH}}/c_{\text{OH}}$

X	K	c_{OR}	c_{OH}	$k' \cdot 10^3$	$k'_I \cdot 10^3$	$k'_{II} \cdot 10^3$	$k_I \cdot 10^2$	$k_{II} \cdot 10^2$
1.000	—	100.0	0.0	5.67	5.67	—	5.67	—
0.894	6.35	98.2	1.8	6.52	6.47	0.05	6.59	2.78
0.802	5.64	95.8	4.2	6.73	6.63	0.10	6.92	2.38
0.718	4.96	92.7	7.3	7.40	7.23	0.17	7.80	2.33
0.660	4.59	89.9	10.1	7.50	7.32	0.18	8.14	1.78
0.400	3.65	70.9	29.1	10.7	9.85	0.85	13.9	2.92
0.310	3.52	61.3	38.7	11.5	10.2	1.30	16.6	3.36
0.230	3.50	51.1	48.9	12.8	10.8	2.00	21.1	4.09
0.162	3.50	40.4	59.6	15.2	11.7	3.50	29.0	5.87
0.101	3.62	28.9	71.1	16.2	10.8	5.40	37.4	7.59
0.047	3.91	16.2	83.8	15.8	7.48	8.32	46.2	9.93
0.022	4.20	8.6	91.4	14.2	4.10	10.1	47.7	11.0
0.011	4.35	4.6	95.4	13.0	2.10	10.9	45.6	11.4
0.000	—	0.0	100.0	11.8	—	11.8	—	11.8

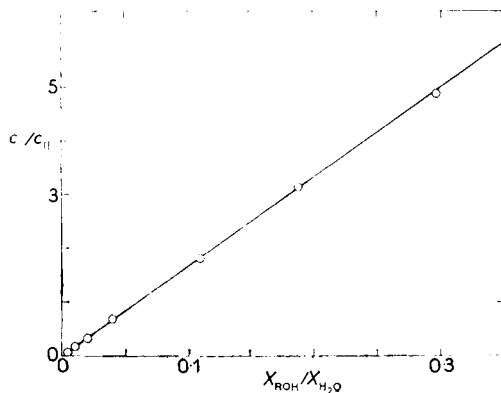


FIG. 4

The dependence of the concentration ratio of monothiourethane *I* to monothiocarbamate *II* on the ratio of mol fractions of methanol and water in the reaction of phenyl isothiocyanate in methanolic alkaline solution ($0.1 \text{ mol dm}^{-3} \text{ NaOH}$)

The Effect of Concentration of Nucleophiles

The c_I/c_{II} ratio, and hence also the ratio of the observed rate constants (Eq. (3)), is practically independent of the hydroxyl ion concentration, as this concentration stands in linear relation to that of the alkoxide ions at the experimental conditions.

At the beginning, the yield of compound *I* increases linearly with increasing alcohol concentration, later it approaches asymptotically the initial concentration of *III* (Fig. 5). The effect of this factor on the rate constants observed is given in Fig. 6 and

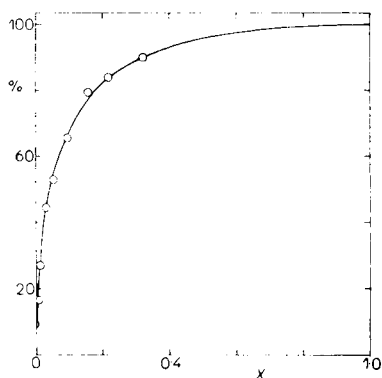


FIG. 5

Dependence of the amount of the mono-thiourethane formed (with respect to isothiocyanate, %) on the mol fraction of methanol in the reaction mixture after finishing the reaction of phenyl isothiocyanate in aqueous-methanolic solution of NaOH at 25°C

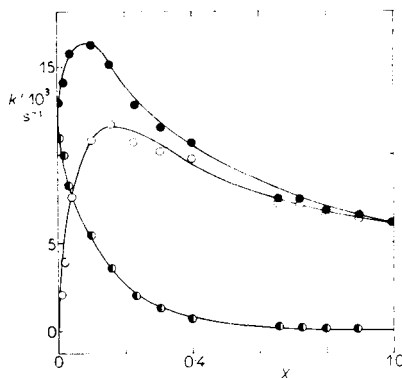


FIG. 6

The dependence of the observed rate constants of the reaction of phenyl isothiocyanate in alkaline ($0.1 \text{ mol dm}^{-3} \text{ NaOH}$) aqueous-methanolic solution on the mol fraction X of methanol: ● k' , ○ k'_I , and ● k'_{II} (all three in s^{-1})

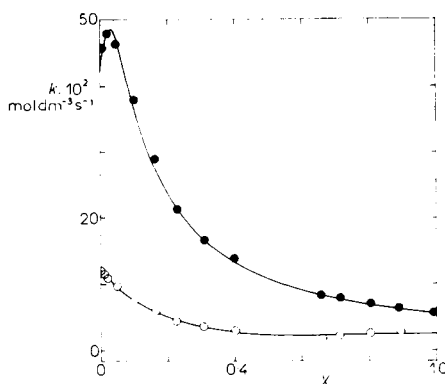


FIG. 7

The dependence of the rate constants of the reaction of phenyl isothiocyanate in alkaline ($0.1 \text{ mol dm}^{-3} \text{ NaOH}$) aqueous-methanolic solution on the mol fraction X of methanol: ● k_I , ○ k_{II} (both in $\text{mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$)

that on the rate constants in Fig. 7. The experimental values are summarized in Table II. It is seen that methanol affects the reaction rate not only as the reactant but also as the reaction medium.

The Effect of Temperature

At a constant ratio of NaOH to alcohol, the yield of monothiourethane *I* decreases with temperature whereas that of monothiocarbamate *II* increases, hence the c_I/c_{II} ratio decreases (Table III). Consequently, also the ratio of the rate constants observed decreases with temperature. This phenomenon is due to a distinct decrease of the value of equilibrium constant *K*, because the ratio of the real rate constants is changed only slightly with temperature (Table III). This can be understood, as both the cases consist in nucleophilic addition to the carbon atom of NCS group. From such measurements it is possible to determine the $E_{II} - E_I$ value (defined by Eq. (5) (ref.¹⁷)) directly without evaluation of kinetics.

$$E_{II} - E_I = E_{II}^0 - E_I^0 - \Delta H \quad (5)$$

E^0 means the activation energy of the reaction with hydroxyl (*II*) and alkoxide ion

TABLE III

The effect of temperature on the concentration ratio of the products *I* and *II* (Scheme 1) in the reaction of benzyl isothiocyanate with methoxide and hydroxyl ions. The reaction mixture: methanol (5 mol dm⁻³), NaOH (0.5 mol dm⁻³), water. The concentration of the monothiourethane *I* was followed spectrophotometrically at 224 nm after finishing the reaction and decomposing the monothiocarbamate *II* by acidification of the reaction mixture to pH 1. The ratio of the rate constants k_I/k_{II} (Scheme 1) calculated from Eq. (4), *K* is the equilibrium constant (Scheme 1) calculated from the value¹¹ found at 25°C with application of the value¹² $\Delta H = -14.64$ kJ mol⁻¹

<i>t</i> , °C	c_I/c_{II}	<i>K</i>	k_I/k_{II}
0	1.98	6.2	2.94
5	1.79	5.5	3.00
10	1.69	4.9	3.17
15	1.50	4.4	3.14
20	1.37	4.0	3.15
25	1.33	3.6	3.40
30	1.15	3.3	3.21
35	1.09	3.0	3.34
40	0.97	2.7	3.31

(I), and ΔH denotes the enthalpy change for the reaction characterized by the equilibrium constant K . For this purpose, Eq. (6) is used.

$$\ln (c_I/c_{II}) = (E_{II} - E_I)/RT + \text{const.} \quad (6)$$

In our case (Table III) the value found was $E_{II} - E_I = 12.36 \text{ kJ mol}^{-1}$, the statistical parameters of Eq. (6) being $n = 9$, $r = 0.994$, $s = 0.027$. $F = 631.1$.

DISCUSSION

From the results presented it follows that the reactivity of primary alcoholic group is comparable with that of amino group^{2,3}, being by roughly three orders lower than that of thiolate anion^{4,5}. Owing to high pK_a values of alcohols, as compared with those of amino groups, the real reactivity will decrease by several orders of magnitude. If for simplification a homogeneous solution of phenyl isothiocyanate of pH 7.4 is considered with equimolecular concentrations of glycine ($pK_a = 9.81$, $k = 0.24 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$)², glutathione ($pK_a = 8.56$, $k = 586 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$)⁵, and methanol ($k'_{RO^-} \sim 0.001 \text{ s}^{-1}$, Fig. 6, extrapolation to zero methanol concentration), then the ratio of the rate constants observed (and, hence, of the reaction products, too) will be $k'_{R-NH_2} : k'_{R-S^-} : k'_{RO^-} = 1 : 4 \cdot 10^4 : 1 \cdot 10^{-5}$. Even if it is considered that the pK_a values are lower for glycols (*e.g.* for glycerol $pK_a = 14.4$, for mannitol $pK_a = 13.29$)¹⁸, the concentration ratio of the isothiocyanate bound to a thiol group to that bound to alcoholic group will be $10^6 - 10^7$. From what was said it follows that the reaction of isothiocyanates with alkoxide ions will make itself felt in biological systems only in special cases, *e.g.* after an accumulation of these compounds in certain substructures due to non-uniform subcellular distribution in a cell.

The reaction of isothiocyanates with alcohols can be utilized for syntheses of the respective monothiourethanes in such cases where preparation of alcoholates in anhydrous media or heating to higher temperatures are excluded.

REFERENCES

1. Drobnic L., Kristian P., Augustin J. in the book: *The Chemistry of Thiocyanate and Isothiocyanate Groups* (S. Patai, Ed.). Wiley, New York 1977.
2. Drobnic L., Augustin J.: *This Journal* 30, 99 (1965).
3. Drobnic L., Augustin J.: *This Journal* 30, 1221 (1965).
4. Drobnic L., Augustin J.: *This Journal* 30, 1618 (1965).
5. Podhradský D., Drobnic L., Kristian P.: *Experientia* 35, 154 (1979).
6. Zahradnik R.: *This Journal* 24, 3407 (1959).
7. Walsh Ch.: *Enzymatic Reaction Mechanism*. Freeman, San Francisco 1979.
8. von Hofmann A. W.: *Chem. Ber.* 2, 116 (1869).
9. Schneider W., Douglas C., Hüllweck G., Steibelt W.: *Chem. Ber.* 47, 1248 (1914).
10. Gockeritz G., Pohloudek-Fabini R.: *Pharmaz. Zentralhalle* 102, 685 (1963).

11. Murto J.: *Alkoxide Equilibria and Kinetics of Alkaline Solvolyses of Methyl Iodide and Some Aromatic Nitro Compounds in Mixed Hydroxylic Solvents*, Ann. Acad. Sci. Fennicae, Series A, II. Chemica, No 117, p. 65. Helsinki 1962.
12. Ref.¹¹, p. 77.
13. Houben-Weyl: *Methoden der organischen Chemie*, Band IX, p. 832. Stuttgart 1955.
14. Brown D. W., Dyson G. M.: J. Chem. Soc. (London) 1931, 3285.
15. Reid E. E.: *Organic Chemistry of Bivalent Sulphur*, Vol. IV. Chemical Publishing Co., New York 1962.
16. Augustín J., Drobnica L., Nemeč P.: *Zborník prác Chemickotechnologickej fakulty SVŠT*, p. 229. Published by Alfa, Bratislava 1978.
17. Murto J.: Acta Chem. Scand. 18, 1029 (1964).
18. Thamsen J.: Acta Chem. Scand. 6, 270 (1952).

Translated by J. Panchartek.