

**REACTIVITY OF ALKYL 2-ISOTHIOCYANATO
AND ω -ISOTHIOCYANATO CARBOXYLATES***L.DROBNICA^a, E.FLOCH^b and P.GEMEINER^{a**}^a Department of Technical Microbiology and Biochemistry and^b Department of Organic Chemistry,

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The reactivity of 21 esters of 2-isothiocyanato and ω -isothiocyanato carboxylic acids in the reaction with glycine, SH^- and OH^- ions was investigated. The kinetics of these nucleophilic addition reactions were studied in systems water-organic solvent at 25°C. The Taft equation was found to hold for the reaction of isothiocyanates with SH^- ions and glycine. The second order rate constants, k_{NH_2} and k_{SH} [$\text{M}^{-1} \text{s}^{-1}$], show that the studied isothiocyanates are more reactive than unsubstituted alkyl isothiocyanates and less reactive than phenyl isothiocyanate.

Isothiocyanates, derived from amino acids, represent a group of compounds interesting from the chemical as well as from the biological point of view. Preparation of these types of compounds containing a free carboxyl in the α -position relative to the isothiocyanato group encounters with difficulties^{1,2} whereas preparation of the corresponding esters is relatively facile³. The interaction between isothiocyanato and alkoxy carbonyl groups in 2-isothiocyanato carboxylates was studied using IR and ¹H-NMR spectroscopy⁴. In order to complete these investigations we decided to study the kinetics of reactions of these compounds with selected nucleophilic reagents. It is assumed that NCS and COOR groups bonded to the same carbon will have a characteristic effect on the rate constants of the studied reactions. The hitherto available data show a high reactivity and at the same time instability, of aroyl isothiocyanates in which the isothiocyanato group is bonded directly to the carbonyl carbon. These compounds are hydrolysed in an aqueous medium to the corresponding carboxylic acids and SCN^- ions².

The subject of this communication is the study of reactivity of alkyl isothiocyanato carboxylates in the reaction with glycine, and SH^- and OH^- ions. The second of these reactions is important also in connection with the elucidation of biological activity of isothiocyanates because this activity is caused mainly by reactions of NCS

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groups with protein as well as non-protein thiol groups of cell components⁵. The studied isothiocyanates can be of interest also as *a*) potentially specific and reversible SH reagents for the study of SH-enzymes⁶; *b*) "reporter" or yardstick groups for structural studies of the active *sites* in SH-enzymes⁶; *c*) bifunctional reagents for preparation of macromolecular polyisothiocyanates^{7,8} and for the subsequent immobilisation of enzymes⁸, as well as for the covalent chromatography of thiols and SH-enzymes⁹, and *d*) new derivatives for the determination of the primary structure of proteins by Edman reaction¹⁰. Data on the reactivity of this series of isothiocyanates in the reaction with nucleophilic reagents simulating some functional groups of enzymes can thus contribute to the above-mentioned exploitations.

TABLE I
UV-Spectral Characteristics of Isothiocyanates

Compound ^a	Dioxane		Methanol		0.2M Borate buffer pH 8.06	
	λ_{\max} nm	log ϵ	λ_{\max} nm	log ϵ	λ_{\max} nm	log ϵ
<i>I</i>	248	3.06	—	—	245	3.04
<i>II</i>	248	3.13	248	2.98	246	3.00
<i>III</i>	248	3.09	—	—	244	3.06
<i>IV</i>	249	3.07	—	—	245	3.06
<i>V</i>	248	3.09	—	—	244	3.06
<i>VI</i>	251	3.29	—	—	245	3.17
<i>VII</i>	248	3.07	249	2.96	246	3.09
<i>VIII</i>	249	3.10	245	3.20	245	3.07
<i>IX</i>	248	3.09	247	3.06	245	3.06
<i>X</i>	249	3.08	245	3.26	246	3.15
<i>XI</i>	—	—	246	3.15	—	—
<i>XII</i>	—	—	245	3.32	—	—
<i>XIII</i>	245	3.12	249	—	249	3.09
<i>XIV</i>	249	3.14	249	—	247	3.11
<i>XV</i>	247	3.06	246	3.31	244	3.36
<i>XVI</i>	247	3.30	248	3.29	245	3.29
<i>XVII</i>	251	3.15	248	3.07	248	3.10
<i>XVIII</i>	229 ^b	3.98	228	4.19	226 ^c	3.99
<i>XIX</i>	224 ^b	4.79	—	—	225 ^{b,c}	4.43
<i>XX</i>	246	2.96	247	2.91	242	2.92
<i>XXI</i>	246	3.04	—	—	234	3.00

^a The structural formulas are given in Table II; ^b other bands: λ_{\max} 311 nm, log ϵ 3.71; ^c absorbs also at 245—250 nm.

RESULTS AND DISCUSSION

The UV absorption maxima of the studied isothiocyanates are situated in the range 247–251 nm, $\log \sim 3$ (Table I). The positions of the bands and their $\log \epsilon$ values are in accord with the results of a UV-spectral study of alkyl isothiocyanates¹¹. As seen from a comparison of spectra, taken in dioxane and in 0.2M borate buffer (pH 8.06), the values of λ_m and $\log \epsilon$ decrease with increasing polarity of the system. The strongest absorption band in the spectra of the compounds XVIII and XIX is situated at lower λ_m , whereas the value of its $\log \epsilon$ is much higher than found for other compounds. On the basis of comparison of λ_m for the compounds XVII, XVIII and XIX this band can be assigned to the substituted benzene chromophore which in the region 245–250 nm obscures the NCS band¹².

TABLE II

UV Spectral Characteristics λ_{\max} , nm, ($\log \epsilon$) of the Compounds $R^1CH(R^3)COOR^2$ and $R^2OOC \cdot (CH_2)_x R^3$ Arising in the Reaction of the Isothiocyanates $R^1CH(NCS)COOR^2$ ($I-XXI$) with Glycine and SH^- Ions

Starting isothiocyanate	R ¹	R ²	R ³	
			NHCSNHCH ₂ COO ⁽⁻⁾ a	NHCSS ⁽⁻⁾ b
I	H	CH ₃	265 (3.76)	288 (4.25)
II	H	C ₂ H ₅	264 (3.76)	288 (4.29)
III	H	n-C ₃ H ₇	—	289 (4.31)
IV	H	i-C ₃ H ₇	—	—
V	H	n-C ₄ H ₉	—	—
VI	H	t-C ₄ H ₉	—	246 (4.12)
VII	CH ₃	C ₂ H ₅	265 (3.70)	289 ^c (4.14)
VIII	C ₂ H ₅	C ₂ H ₅	265 (3.65)	290 (3.55)
IX	i-C ₃ H ₇	C ₂ H ₅	270 (4.08)	288 (3.99)
X	i-C ₄ H ₉	C ₂ H ₅	270 (3.60)	288 (4.01)
XI	s-C ₄ H ₉	C ₂ H ₅	268 (3.78)	—
XII	n-C ₄ H ₉	C ₂ H ₅	265 (4.13)	288 (4.03)
XIII	C ₂ H ₅ OOCCH ₂	C ₂ H ₅	270 (3.68)	—
XIV	C ₂ H ₅ O ₂ C(CH ₂) ₂	C ₂ H ₅	268 (3.89)	—
XV	SCN(CH ₂) ₃	C ₂ H ₅	268 (3.99)	—
XVI	SCN(CH ₂) ₄	C ₂ H ₅	264 (3.87)	—
XVII	C ₆ H ₅ CH ₂	C ₂ H ₅	268 (4.22)	—
XVIII	4-HOC ₆ H ₄ CH ₂	C ₂ H ₅	267 (4.12)	290 ^d (3.48)
XIX	4-HOC ₆ H ₂ I ₂ CH ₂	C ₂ H ₅	—	—
XX	x = 2	C ₂ H ₅	278 (3.30)	286 (4.27)
XXI	x = 3	C ₂ H ₅	278 (3.30)	286 (4.24)

^a Products of reactions with glycine; ^b products of reactions with SH^- ions; ^c further maximum λ_{\max} 330 nm (3.75); ^d further maximum at λ_{\max} 332 (3.81).

Table II summarises the UV-spectral data of the products of reaction of the studied isothiocyanates with glycine and SH^- ions, *i.e.* of N-substituted thiocarbamoylaminoacetic acids and the corresponding salts of dithiocarbamic acids, respectively. In addition to the above-mentioned bands, the spectra of N-substituted thiocarbamoyl-acetic acids exhibit bands in the region of shorter wavelengths¹³, in our case at 230–240 nm. The values λ_m and $\log \epsilon$, found for the dithiocarbamates, correspond to the $\text{NH}-\text{CS}-\text{S}^{(-)}$ grouping^{14,15}. However, these compounds exhibit also other characteristic bands the assignment of which is discussed in detail elsewhere^{16,17}.

The kinetic data for the reactions of the isothiocyanates *I–XXI* with glycine and SH^- ions are summarised in Table III. The reactivity is characterised by second order rate constants $k(\text{M}^{-1}\text{s}^{-1})$ which serve as a basis for discussion of the relations between structure and reactivity. This holds also for the isothiocyanates *XV* and *XVI* in which the presence of a second NCS group did not change the order of reaction.

TABLE III

Rate Constants for the Reactions of 2-Isothiocyanato and ω -Isothiocyanato Carboxylic Acids with Glycine and SH^- Ions at 25°C

Compound	k_{NH_2}		k_{SH}		σ^{*a}
	$10^4 k_{\text{obs}}$ s^{-1}	$10^2 k$ $\text{M}^{-1} \text{s}^{-1}$	$10^3 k_{\text{obs}}$ s^{-1}	k $\text{M}^{-1} \text{s}^{-1}$	
<i>I</i>	—	—	9.10	9.42	—
<i>II</i>	36.33	18.10	7.50	7.77	0.49
<i>III</i>	—	—	7.07	7.33	—
<i>V</i>	—	—	26.00	6.80	—
<i>VII</i>	17.00	7.58	7.73	4.02	0.00
<i>VIII</i>	7.70	3.85	8.88	2.29	–0.10
<i>IX</i>	7.40	3.70	8.28	2.16	–0.19
<i>X</i>	8.83	4.42	11.40	2.97	–0.125
<i>XI</i>	4.37	2.18	4.80	2.50	–0.21
<i>XII</i>	6.87	3.43	—	—	–0.13
<i>XIII</i>	44.17	22.08	—	—	0.60
<i>XIV</i>	18.57	9.28	—	—	—
<i>XV</i>	15.33	7.67	—	—	—
<i>XVI</i>	14.13	7.07	—	—	—
<i>XVII</i>	14.77	7.38	13.65	3.55	0.22
<i>XVIII</i>	9.80	4.90	—	—	—
<i>XX</i>	11.67	5.83	9.68	6.75	—
<i>XXI</i>	—	—	46.50	6.01	—

^a Taken from ref.¹⁸.

Under the given reaction conditions obviously only the analytical concentration of the NCS groups was changed but the reactivity of the compound in question was not affected. Analogous results have been found in kinetic studies of reactions of polyisothiocyanates with mercapto and amino compounds^{7,9}.

It follows from the results that, in the reaction with glycine or HS^- ion, the reactivity of the studied isothiocyanato analogues of amino acids increases with the decreasing density of π -electrons on the carbon of the NCS group which is achieved by variation of the substituent R in $\text{RCH}(\text{NCS})\text{COOC}_2\text{H}_5$. The reactivity in the reaction with glycine and HS^- ions corresponds to the Taft equation $\log k_i/k_0 = \rho\sigma^*$, the correlation coefficients being $r = 0.96$ and 0.92 , respectively (Table IV). This represents the first case of a linear free energy relationship in a series of aliphatic isothiocyanates. The relatively low slope values in these relationships (0.72 and 1.08) remind the slope values in the Hammett equation used for the 4-substituted phenyl isothiocyanates and various thiols¹⁹. In both cases we can speak about a low sensitivity of the reaction center to the variation of the nature of substituent¹⁸ R as well as about the low charge of the activated complex²⁰.

TABLE IV

Taft Equations for the Reaction of Isothiocyanates with Nucleophilic Reagents

Nucleophile	ρ	$\log k_0$	r	n^a
NH_2 (glycine)	1.08 ± 0.11	-1.29	0.96	9
SH^-	0.72 ± 0.14	0.49	0.92	7

^a Number of correlated values.

TABLE V

Rate Constants for Alkaline Hydrolysis (k_{NH_2}) and for Addition of OH^- Ions (k_{NCS}) to the Compounds $\text{R}^1\text{CH}(\text{R}^3)\text{COOR}^2$ at 25°C

R^1	R^2	$\text{R}^3k, \text{M}^{-1} \text{s}^{-1}$		$k_{\text{NH}_2}/k_{\text{NCS}}$
		$\text{NH}_2^{a,b}$	NCS	
H	CH_3	1.28_3	0.06_7	19.2
H	C_2H_5	0.63_3	0.05_8	10.9
$i\text{-C}_4\text{H}_9$	C_2H_5	0.18_3	0.02_4	7.6

^a $I = 0.1$, according to ref.²²; ^b $\sigma_1 = 0.60$ for NH_2 , $\sigma_1 = 0.61$ for NCS ²³.

We investigated also the reactivity of isothiocyanates in the reaction with OH^- ions. The reaction products exhibited in all cases one absorption band in the region above 220 nm, λ_m ranging from 227 to 230 nm ($\log \epsilon \sim 4$). These values correspond well to the previous data on N-monoalkyl monothiocarbamates¹¹. The reaction of isothiocyanates with OH^- ions is, however, more complicated than the reaction of unsubstituted alkyl isothiocyanates^{11,21}. This is shown by the low value of the correlation coefficient of the Taft equation for the reaction of the studied isothiocyanates with OH^- ions ($r = 0.63$). Although under given experimental conditions the reaction was in all cases of the first order, the obtained kinetic data cannot be regarded as unequivocal because the measured parameter was the concentration change of the product. On the basis of the literature data²³ it can be expected that the rate of alkaline hydrolysis of the studied isothiocyanato compounds will be almost the same as the rate of methyl and ethyl esters of amino acids²². This hydrolysis will be fast enough to compete with the addition of OH^- ions (Table V). We used relatively high concentrations of OH^- ions in order to suppress the decomposition of monothiocarbamates into amines during the measurement²⁴. Also dithiocarbamates decompose to amines at lower pH values²⁵. The reaction of isothiocyanates with OH^- ions affords in all cases monothiocarbamates, as found by UV spectral analysis of the reaction products. This indicates that compounds of the general formula $\text{RCH}(\text{NHCSO}^{(-)})\text{COO}^{(-)}$ can exist, in which an $-\text{NH}-\text{CS}-\text{O}^{(-)}$ group and an ionized carboxyl group are bonded to the same carbon atom.

The reactivity of nucleophilic reagents in reactions with isothiocyanates is in accord with the existing experimental results^{26,27} as well as with theoretical reasons for the differences in their nucleophilicity²⁸. In all isothiocyanato derivatives the k_{OH} values are lower than k_{NH_2} (for glycine) which in turn are much lower than the values of

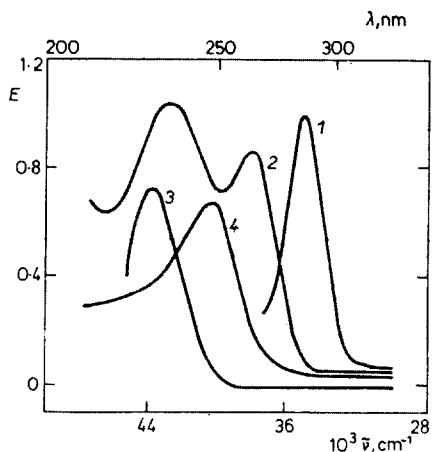


FIG. 1

UV Absorption Spectra of the Products of Reaction of Ethyl 2-Isothiocyanatoacetate

1 with Na_2S at pH 8.06 in 0.2M borate buffer, c 50 μM , d 10 mm; 2 with glycine at pH 9.80 in 0.2M borate buffer pH 8.06, c 50 μM , d 10 mm; 3 with 0.25M-NaOH, c 50 μM , d 10 mm; 4 UV spectrum of ethyl 2-isothiocyanatoacetate in dioxane, c 1.0 mM, d 5 mm.

k_{SH} . In order to compare the reactivity of the studied isothiocyanates with the reactivity of aryl isothiocyanates, following rate constants $k(M^{-1}s^{-1})$ for the reaction of phenyl isothiocyanate with various nucleophilic reagents can be mentioned: OH^- ions 0.12, butylamine 0.30, glycine 0.24, HS^- ions 13.2, mercaptoacetic acid 1990, 2-mercaptoethylamine 1170, N-acetylcysteine 2000, reduced glutathione 580 (ref.^{19,29}). As seen from Table III, even the most reactive 2-isothiocyanato carboxylates studied do not reach the reactivity of phenyl isothiocyanate, they are, however, more reactive than phenyl isothiocyanate derivatives containing an electron-donating substituent, e.g. 4-methoxyphenyl isothiocyanate, or than derivatives of an aralkyl type, e.g. benzyl isothiocyanate.

2-Isothiocyanato carboxylic acids are more reactive than their ω -isothiocyanato analogues the reactivity of which reminds alkyl isothiocyanates^{21,30}. This supports the conclusions obtained from spectral studies of aliphatic isothiocyanates containing a carboxyl group which is sufficiently remote from the NCS group.

EXPERIMENTAL

Chemicals. Esters of 2-isothiocyanato and ω -isothiocyanato carboxylic acids were prepared according to ref.³. Glycine was a Reanal Budapest product, 2,4,6-trinitrobenzenesulphonic acid was purchased from Serva, Feinbiochemica GmbH, Heidelberg other chemicals from Lachema, Brno. All compounds were of analytical purity.

Methods. The UV-absorption spectra of the isothiocyanates were measured in dioxane, methanol and in 0.2M borate buffer, pH 8.06. The UV absorption spectra of products of the reaction of isothiocyanates with OH^- and SH^- ions and with glycine were measured in the corresponding reaction mixtures after the reactions had been completed. Monothiocarbamates were measured in 25–50 μM solutions in 0.25M-NaOH, dithiocarbamates as 25–50 μM solutions in 0.2M borate buffer (pH 8.06), and N-substituted thiocarbamoylaminoacetic acids as 37.5 to 75.0 μM solutions in 0.2M borate buffer (pH 9.80). In the case of some dithiocarbamates the exact measurement of the first absorption band (246–250 nm) was not possible because of a high absorption of SH^- ions in this region.

The kinetics of the reactions of isothiocyanates with OH^- and SH^- ions and with glycine were followed spectrophotometrically at $25 \pm 0.2^\circ C$. The reaction mixtures contained at least 20fold excess of the nucleophile. In most cases the end extinction value was constant at least during five half-lives of the reaction. The respective starting concentrations of isothiocyanates, OH^- ions, sodium sulphide and glycine were 25–150 μM , 125 mM, 0.16–1.25 mM and 1.5 mM. The isothiocyanates were dissolved in dioxane or methanol, sodium sulphide and glycine in water. The buffered reaction mixtures were prepared using 0.2M borate buffers of pH 8.06 and 9.80, made from 0.2M boric acid and 0.1M-NaOH. The reaction of isothiocyanates with OH^- ions was followed in mixtures of the following composition: 10.0 ml of 0.25M-NaOH, 9.9 ml of dioxane and 0.1 ml of 5–10 mM solution of isothiocyanate in dioxane. The reaction of isothiocyanates with SH^- ions was studied in a mixture of 3.69 ml of 0.2M borate buffer (pH 8.06), 0.19 ml of dioxane, 0.10 ml of 6.4–50.0 mM Na_2S and 0.01 ml of 20 mM isothiocyanate in methanol. The reaction with glycine was performed in a mixture of 10.0 ml of methanol, 7.8 ml of 0.2M borate buffer (pH 9.80), 2.0 ml of 15 mM glycine solution and 0.2 ml of 15 mM isothiocyanate in methanol.

The UV absorption spectra in the region 220–360 nm, as well as the kinetic measurements,

were measured on a recording spectrophotometer Specord UV VIS, (Zeiss Jena) at $25 \pm 0.2^\circ\text{C}$. The pH of the buffers was adjusted using an OP-205 Radelkis (Budapest) pH-meter; accuracy ± 0.01 .

The first order rate constants, k_{obs} (s^{-1}), were calculated according to ref.¹⁹. In all cases the relationships were linear at least till two half-lives of the reaction. The rate constants, k_{obs} , represent arithmetic means of four measurements; the deviation from the mean value was not higher than 5%. The second order rate constants k ($\text{M}^{-1} \text{s}^{-1}$) were calculated from the values of k_{obs} for which the half-life of the reaction was 1.0–1.5 min. The rate constants k were then calculated using the equation $k = k_{\text{obs}}(K_a + c_{\text{H}^+})/(c_0 K_a)$, where K_a (M^{-1}) is the dissociation constant of the nucleophilic reagent, c_0 (M) is its analytical concentration and c_{H^+} (M) is the concentration of H^+ ions, calculated from the pH-meter data. The concentration of OH^- ions was determined acidimetrically, the concentration of sodium sulphide spectrophotometrically or iodometrically according to ref.¹⁹, and the concentration of glycine spectrophotometrically after the reaction with 2,4,6-trinitrobenzenesulphonic acid³¹. The dissociation constants K_a for OH^- (13.99), for SH^- (7.10) and for glycine (9.78) were taken from ref.¹⁹.

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