KINETICS OF DECOMPOSITION AND DISSOCIATION CONSTANTS OF THE S-ESTERS OF N-MONOSUBSTITUTED DITHIOCARBAMIC ACIDS

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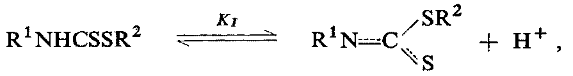
S-esters of N-monosubstituted dithiocarbamic acids (EDTC) of the general formula $R^1NHCSSR^2$ (*Ia*) in alkaline solutions decompose to thiols R^2SH and isothiocyanates R^1NCS, which further react with OH^- ions to corresponding monothiocarbamates. This work gives evidence that a prerequisite of alkaline decomposition is the dissociation of *Ia* to unstable form $R^1N{=}C\genfrac{}{}{0pt}{}{\diagup SR^2}{\diagdown S}$ (*Ib*). The pK_a values of the equilibrium $Ia \rightleftarrows Ib + H^+$ were determined by a modification of the kinetic and spectrophotometric method. Using the pK_a values and the first-order rate constants of the decomposition of *Ib*, the structure-stability relationship for EDTC is discussed.

The S-esters of N-monosubstituted dithiocarbamic acids (EDTC, *Ia*)* represent a class of substances which may arise by the nucleophilic addition reactions of isothiocyanates (ITC) with mercapto compounds including proteins. It is possible to a great extent to explain the biological activity of ITC on account of their reaction with SH groups of different enzymes. On the other hand, the compounds of general formula *Ia*, especially provided R^2 is a low-molecular substituent, also represent a vast class of the biologically active substances called mustard oil formers. These substances are able under physiological conditions to split off ITC functioning as proper effective species. Besides EDTC, the N-monosubstituted dithiocarbamic acids and their salts (DTC) belong among these substances. As to EDTC, their decomposition in aqueous medium yielding the corresponding ITC and mercapto compounds has not been studied from the quantitative point of view yet.

On the basis of papers[1-5] the subsequent reaction scheme may be assumed in aqueous buffered medium for the dissociation and decomposition of EDTC and other consecutive reactions:



$$\underset{Ia}{R^1NHCSSR^2} \xrightleftharpoons{K_I} \underset{Ib}{R^1N{=}C\genfrac{}{}{0pt}{}{\diagup SR^2}{\diagdown S}} + H^+ , \qquad (A)$$

Ia *Ib*

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* Abbreviations: DTC, N-monosubstituted dithiocarbamic acid or its salt; EDTC, S-ester of N-monosubstituted dithiocarbamic acid; ITC, isothiocyanate; MTC, N-monosubstituted monothiocarbamic acid or its salt.

$$Ib \underset{k_{-Ib}}{\overset{k_{+Ib}}{\rightleftharpoons}} \underset{II}{R^1NCS} + \underset{IIIb}{R^2S^-}\,, \qquad (B)$$

$$II + OH^- \xrightarrow{k_{OH^-}} \underset{IV}{R^1NHC\overset{O}{\underset{S}{\lessgtr}}}\,, \qquad (C)$$

$$\underset{IIIb}{R^2S^-} + H^+ \overset{K_{III}}{\rightleftharpoons} \underset{IIIa}{R^2SH}\,. \qquad (D)$$

It is known that the decomposition of EDTC takes place in alkaline medium[6,7] and the stability of EDTC depends[8,9] on the character of the substituents R^1, R^2. It is taken for granted that under convenient conditions the decomposition of EDTC to products *II* and *IIIb* proceeds through *Ib*. Up to the present time, no direct data have been available which would prove structure *Ib* as a decomposing form of EDTC. The possibility of existence *Ib* has been indicated by the results obtained from the study of the reactivity of ITC with respect to various mercapto compounds[1,2] in the course of which the rate constants $k_{-\mathrm{Ib}}$ were determined. A contribution to the proper solution of the problem could be given by determining the values of dissociation constants K_{I}. Because of a unsteadiness of EDTC it is not possible to determine K_{I} by usual methods. This paper presents the values of the dissociation constants K_{I} of different types of the EDTC determined on the basis of kinetic investigations and spectrophotometric measurements.

EXPERIMENTAL

Chemicals. The commercial thiols and sodium sulphide were purified and their analytical concentrations was determined according to our following paper[2]. Similarly, the data concerning synthesis and purification of ITC were published in that paper. The S-propyl esters N-phenyl- and N-benzyl dithiocarbamic acid[10-13], 3-benzyl rhodanine[14,15], N-phenyl and N-benzyl dithiocarbamyl glutathione[16] and the sodium salt of N-phenyl dithiocarbamic acid were prepared according to references cited. N-phenyl and N-benzyl monothiocarbamic acid (MTC) were prepared according to (ref.[3]). For the preparation of stock solutions ITC, EDTC and 3-benzyl rhodanine were dissolved in methanol, sodium sulphide in distilled water, thiols in methanol or distilled water, and MTC in 0·1M sodium hydroxide. The spectrophotometric as well as kinetic measurements were performed with the buffered reaction mixtures containing the Sörensen 0·2M phosphate buffer solution (pH range 6·8—8·0), the Sörensen 0·2M borate buffer solution (pH range 8·0 to 11·9) or the 0·2M hydroxide-chloride buffer solution (pH range 12·0—13·0) (ref.[17]). The value of the ionic strengths of the buffered reaction mixtures was 0·1. The solvents were spectrally pure, the buffer constituents were A.G. chemicals.

Spectrophotometric determination of pK_{I} *values.* Method *A*. To the buffered solutions thermostated at 25°C we added 5 mM solution of the substances studied in the amount of 1 vol.% of the buffer solution and after a constant period of time the UV absorption spectrum in the region 220—350 nm was recorded on a spectrophotometer Specord UV-VIS, Zeiss (Fig. 1*b*). The buffers were adjusted on pH-meter Radelkis, Hungary with the accuracy of ±0·01. Method *B*. It was a measurement like method *A* with the difference that the studied EDTC were prepared by the addition reaction of ITC with thiol which took place immediately after the addition of these substances into the corresponding buffer solutions in the spectrophotometric cell. In order to eliminate the reverse reaction (*B*), the reaction equilibrium was entirely shifted by the addition of 100—400 fold excess thiol with respect to ITC. The concentrations of thiol in reaction mixture necessary for a rapid completion of addition reaction and providing the stability of the product of addition reaction were calculated from the rate constants of the reactions of individual ITC and thiols[1,2,18]. The investigated substances were stable at least for 10 minutes and the reaction was completed in the whole pH range of the buffer solutions used earlier than 30 seconds after mixing ITC and thiol. The methanolic solution of 5 mM ITC was added to the buffer solutions thermostated at 25°C in the amount of 1 vol.% of these solutions. After the completion of addition reaction checked spectrophotometrically the UV absorption spectra were recorded (Fig. 1*a*).

Kinetic determination of pK_I *values*. Method C_1. This method is based on the investigation of the decomposition rate of the substances studied and excludes the effect of equilibrium state (Eq. (*B*)) by the removal ITC from the reaction mixture. One percent methanolic solution of the substances studied (1·5—5·0 mM) was added to the buffer solutions thermostated at 25°C which contained sodium sulphide (1·5—75·0 mM). Sodium sulphide reacted with ITC practically immediatelly, irreversibly and quantitativelly. The concentration of non-decomposed EDTC was

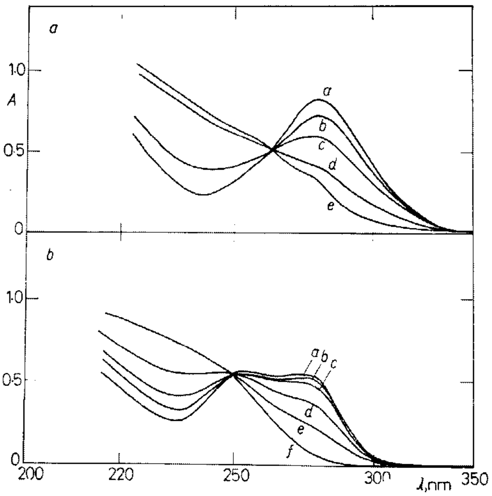

FIG. 1

UV Spectra of (1*a*) the S-(2-Hydroxyethyl) Ester of N-Phenyl Dithiocarbamic Acid (50 μM) Prepared by the Reaction of Phenyl Isothiocyanate (50 μM) with 2-Mercaptoethanol (1·25 mM) Directly in the Measured Solutions and (1*b*) of the S-Propyl Ester of N-Benzyl Dithiocarbamic Acid (50 μM)

a (Curves: *a* pH 6·0, *b* pH 8·0, *c* pH 9·0, *d* pH 10·0, e pH 11·0, *d* = 10 mm) *b* (Curves: *a* pH 7·0—9·0, *b* pH 10·0, *c* pH 11·0, *d* 11·5, e pH 12·0, *f* pH 13·0, *d* = 10 mm).

measured spectrophotometrically in regular time intervals on Specord UV-VIS. The concentrations were estimated from the extinction value of solution at a convenient wavelength.

Method C_2. The samples were extracted by equal volume of cyclohexane and the concentration of EDTC at a given moment was found out by measuring the extinction of cyclohexane layer in the absorption maximum of EDTC. The partition coefficients determined at 25°C in system cyclohexan–water for investigated EDTC were higher as 10^3 and for DTC less as 10^{-3} (used method was described earlier[4]).

Evaluation of the results. Using the results of spectrophotometric methods the constants K_I were calculated according to the Eq. (*1*) (ref.[19]):

$$E_{obs} = (1/K_I)\,[(E_a - E_{obs})\,c_{H^+}] + E_b\,. \qquad (1)$$

The values E_a and E_b stand for the extinction of form *Ia* and *Ib* respectively, E_{obs} denotes the extinction of a mixture *Ia* + *Ib*, K_I(M^{-1}) is the dissociation constant searched for, and c_{H^+}(M) is the concentration of hydrogen ions. The plot of the extinctions found against pH or $(E_a - E_{obs})\,.\,c_{H^+}$ necessary for the calculation K_I is presented as an example in Fig. 2*a* and 2*b*.

The rate constants of the decomposition of EDTC (Eq. (*B*)) were calculated in a similar way as in the next paper[2]. All rate constants thus calculated corresponded to the reactions of first order. The rate constant at least of one of both the forms participating in the dissociation equilibrium has to be measurable and it must be sure that the rate constant thus measured corresponds only to the investigated decomposition, *i.e.* that it is not a composed rate constant of the equilibrium system involving the decomposition reaction. Provided these conditions* are

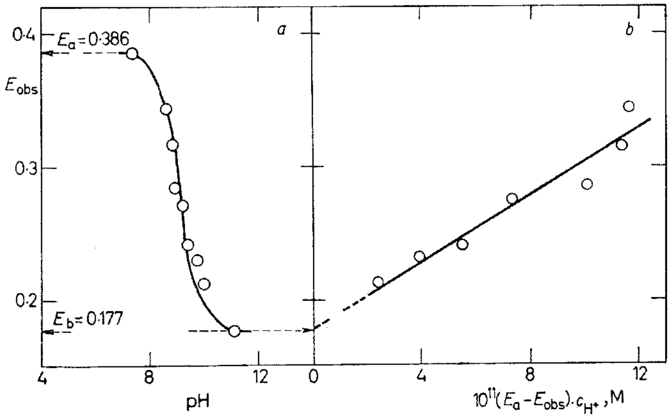

FIG. 2

Spectrophotometric Determination of pK_I of the S-(2-Hydroxyethyl) Ester of N-(4-Bromophenyl) Dithiocarbamic Acid (31·3 μM) by Method *B*

The substance was prepared by the reaction of 4-bromophenyl isothiocyanate (31·3 μM) with 2-mercaptoethanol (1·25 mM) directly in the reaction mixture. *a* E_{obs} as a function of pH, *b* the plot according to Eq. (*1*). More detailed data in Table I.

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* The rates of the dissociation reactions were considered to be infinitely high[20].

TABLE I

The Values of pK_I, k_{+Ia}, and k_{+Ib} of the S-Esters of N-Monosubstituted Dithiocarbamic Acids

No	$R^1NHCSSR^2$		pK_I^a	10^4k_{+Ia} s^{-1}
	R^1	R^2		
I	p-BrC_6H_4	C_2H_4OH	8·93 ± 0·04	—
II	C_6H_5	n-C_3H_7	9·11 ± 0·02	0·10
			9·17 ± 0·03	—
III	C_6H_5	C_2H_4OH	9·20 ± 0·04	—
IV	p-$BrC_6H_4CH_2$	C_2H_4OH	11·58 ± 0·28	—
V	$C_6H_5CH_2$	C_2H_4OH	11·59 ± 0·04	—
VI	$C_6H_5CH_2$	n-C_3H_7	11·88 ± 0·02	3·00
			11·87 ± 0·02	—
VII	$C_6H_5CH_2$	CH_2COOH	11·99 ± 0·02^i	10·70
VIII	C_6H_5	glutathione	9·17 ± 0·08	—
IX	$C_6H_5CH_2$	glutathione	12·05 ± 0·10	—

fulfilled and the decomposition is a first order reaction, it is possible to determine kinetically K_I according to modified form of the Henderson–Hasselbach equation[21]. By rearranging this equation we obtain a form which is more convenient for the results of our measurements:

$$k_{obs} = (-1/K_I)\,[(k_{obs} - k_{+Ia})\,c_{H^+}] + k_{+Ib}\,, \tag{2}$$

$$k_{obs} = -\,K_I[(k_{+Ib} - k_{oba})/c_H{}^+] + k_{+Ia}\,,$$

where k_{+Ia} and k_{+Ib} (s^{-1}) are the rate constants of the decomposition of *Ia* resp. *Ib* and k_{obs} (s^{-1}) c_{H^+} and K_I are the rate constant of the decomposition of a mixture containing *Ia* and *Ib* for a given pH, the concentration of hydrogen ions, and the dissociation constant searched for, respectively. The experimental data k_{obs}, k_{+Ia}, and k_{+Ib} were inserted in Eq. (*2*) or (*3*) and the values of K_I, k_{+Ib}, and k_{+Ia} were calculated by the least square method. The plot of k_{obs} against pH or other variables in the sense of Eq. (*2*) and (*3*) is presented as illustrations in Fig. 3*a* and 3*b*.

The mole fractions x_r/a of decomposed EDTC were calculated either from the values of the extinction of reaction mixtures found by the kinetic measurements (method C_1) or from the rate constants listed in Table I, and rate constants k_{-Ib} given in paper[2] which has been measured under identical conditions. The following relationship were used for the calculation

$$x_r/a = (E_0 - E_r)/E_0\,, \tag{4}$$

$$x_r/a = (0{\cdot}5a)\,(A^{0{\cdot}5} - A)\,(A + 4a)^{0{\cdot}5}\,, \tag{5}$$

$$A = k_{obs}K_I/[K_I + c_{H^+})\,k_{-Ib}]\,, \tag{5a}$$

TABLE I

(Continued)

10^4k_{+Ib} s^{-1}	Method	λ^b nm	$pK_{NH_2}{}^c$	$pK_{SH}{}^d$	$k_{-Ib}{}^e$ $M^{-1}s^{-1}$	n^f
—	*B*	280	3·87	9·46	1 100	7
25·5	C_2	285	4·60	10·65[g]	773[h]	7
—	*A*	280	—	—	—	6
—	*B*	276	4·60	9·48	607	7
—	*B*	272	—	9·48	—	4
—	*B*	280	9·34	9·48	228	4
92·7	C_2	252	9·34	10·65[g]	252	6
—	*A*	273	—	—	—	7
106·0	C_2	285	9·34	10·11	1 900	6
—	*B*	275	4·60	8·75[j]	670	6
—	*B*	270	9·34	8·75[j]	231	4

[a] The values of correlation coefficients are higher than 0·9 except for the line *4*; [b] wavelengths used for measurements; [c] the pK_a values of corresponding R^1NH_2 from ref.[24]; [d] the pK_a values of corresponding R^2SH according to ref.[2]; [e] from ref.[2]; [f] the number of pH values used for the calculation of K_I, k_{+Ia}, k_{+Ib}; [g] determined by method *A*; [h] for the reaction of phenyl isothiocyanate with ethanethiol according to ref.[2]; [i] substance prepared by adding 3-benzyl rhodanine into reaction mixture used for kinetic investigations with irreversible and quantitative opening ring according to ref.[14,15]; [j] from ref.[25].

where E_0 is the extrapolated value of the extinction at t_0 (from extinction-time relationship), E_r is the extinction in equilibrium state, *i.e.* in the moment when the slope of the curve expressing $\log (E_0 - E_t)$ as a function of time changes abruptly (Fig. 4), x_r is the concentration of decomposed EDTC at equilibrium, and a is the concentration of EDTC at time t_0. Eq. (*5*) is a modification of the relationship between time and the equilibrium concentration[22,23] of the EDTC decomposed or of one of the decomposition products in reaction (*B*) under simplifying conditions, *i.e.* provided the pH of reaction system is higher than the pK_{III} of arising thiol which could be achieved in the case of the S-propyl ester of N-benzyl dithiocarbamic acid (Table II).

RESULTS AND DISCUSSION

The method *A* of the determination of pK_a values (Fig. 1*b*) could not be used for the majority of the investigated EDTC, because these substances rather decompose. The modification of this method (method *B*) makes possible to determine the pK_a values even of very unstable substances (Table I). The kinetic method of pK_a determination can be, in principle, also used for the determination of the pK_a values of EDTC by measuring the dependence of the rate constant k_{obs} of Eq. (*B*) on pH. As stated before, reaction (*B*) is reversible and tends to equilibrium state. The values of the molar fractions x_r/a of S-propylester of N-benzyl dithiocarbamic acid show that the equilibrium cannot be neglected (Table II). The rate constants calculated from the initial decomposition rates when the effect of reverse reaction could be neglected were charged at the technique used with a considerable error, especially in the case of extremely unstable substances. For instance, it was found for the S-propylester of N-benzyl dithiocarbamic acid at pH 12 that $k_{obs} = 1{\cdot}53 \,.\, 10^{-2}\, s^{-1}$ ($t_{0.5} =$ $= 45$ s). Moreover, the situation is complicated by the fact that in alkaline medium the consecutive addition reaction (*C*) of the arising ITC with OH^- ions starts to be effective. The existence of the MTC (Eq. (*D*)) thus formed is confirmed by the identity of the UV absorption spectra of EDTC after decomposition in 0·1M-NaOH with the UV absorption spectra of corresponding MTC. The rate of the reaction of arising ITC with OH^- ions is a function of the concentration of OH^- ions as indicated by the values of k'_{OH} of reaction (*C*) (Table II). Notwithstanding even at high values

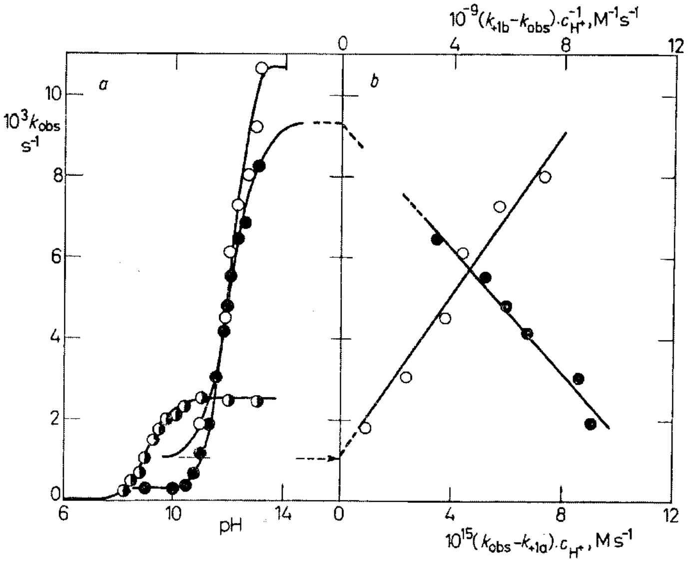

FIG. 3

Determination of pK_1 from Kinetic Measurements

Decomposition of the S-propyl ester of N-benzyl (● — ●), S-carboxymethyl ester of N-benzyl-(○ — ○), and S-propyl ester of N-phenyldithiocarbamic acids (◑ — ◑) (25—50 μM) in the presence of sodium sulfide (2·5—7·5 mM) in buffer solutions of ionic strength 0·1 at 25°C. *a* k_{obs} as a function of pH; *b* the plot according to Eq. (*2*) (● — ●, lower abscissa scale) and Eq. (*3*) (○ — ○, upper abscissa scale). More detailed data in Table I.

of pH, the rate of reaction (*C*) is not sufficient for an instantaneous shift in the equilibrium of reaction (*B*). This follows from the observations concerning the values of k_{obs} for the alkaline decomposition of the S-propyl ester of N-benzyl dithiocarbamic acid (Table II, column 1 and 2), k'_{OH} (Table II), and k_{obs} for the alkaline decomposi-

TABLE II

Rate Constants k_{obs}, k'_{OH} and Equilibrium Concentrations of the S-Propyl Ester of N-Benzyl Dithiocarbamic Acid (as Mole Fractions x_r/a) Decomposed in Alkaline Medium

Initial concentration of EDTC 50 μM, temperature 25°C.

pH	$10^5 k_{obs}$, s^{-1}			$10^5 k'_{OH}{}^d$ s^{-1}	x_r/a	
	1^d	2^b	3^c		1^e	2^f
9·5	1·4	—	30·0	0·3	—	—
11·5	8·7	8·9	218·0	31·4	0·27	0·29
12·0	19·7	20·2	550·0	99·0	0·33	0·48
13·0	277·0	295·0	825·0	990·0	—	—

[a] Measured in the absence of sodium sulfide without extraction at the wavelength of 273 nm; [b] measured in the absence of the sulfide with extraction; [c] measured by the method C_2, the concentration of the sulfide 7·5 mM, the wavelength 252 nm; [d] calculated according to $k_{OH} = k_{OH} \cdot c_{OH^-}$, the value $k_{OH} = 0{\cdot}119\text{M}^{-1}\,\text{s}^{-1}$ taken from ref.[3]; [e,f] calculated according to Eqs (*4*) and (*5*).

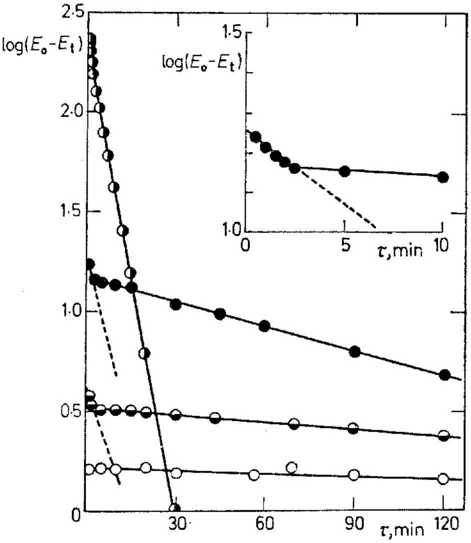

FIG. 4

Semilogarithmic Relationship between Time and the Change in Extinction for an Alkaline Decomposition of the S-Propyl Ester of N-Benzyl Dithiocarbamic Acid (50 μM)

○—○ pH 9·5, ⊖—⊖ pH 11·5, ●—● pH 12·0, ◑—◑ pH 13·0. Ionic strength 0·1, wavelength λ 273 nm, temperature 25°C. Direct measurements of the extinction of the reaction mixture in the absence of sodium sulphide. Full lines found experimentally, dashed lines represent the course expected on the basis of the initial rates found.

tion of the above mentioned EDTC (Table II, column 3). In order to eliminate the effect of reverse reaction (*B*), we selected some convenient nucleophilic agents which would be able to remove the arising ITC quantitatively, irreversibly and instantaneously from the reaction mixture and thus shift the equilibrium of the reaction (*B*)) entirely to the benefit of EDTC decomposition. It appeared that the most convenient way was to bind the arising ITC by means of the sulphide present in the reaction mixture which resulted in the formation of the corresponding salt of dithiocarbamic acid. The comparison of k_{obs} for the decomposition of EDTC with the apparent rate constants of the reaction of ITC with sulphide ion at different concentrations of sulphide showed clearly that the sulphide concentrations (5·0 – 7·5 mM) used for the determination of k_{obs} were sufficient for providing total and permanent shift in equilibrium (Table III). Under given conditions, DTC arising in the reaction mixtures was stable. However, since DTC, too, shows a characteristic absorption maximum of about equal intensity in the same wavelength as EDTC studied, the

TABLE III

The Values of the Rate Constants k_{obs} for the Decomposition of EDTC

$R^1NHCSSR^2$		pH	^{c}EDTC μM	^{c}HS$^-$ mM	10^3k_{obs}[a] s^{-1}	$10^3k'$[b] s^{-1}
R^1	R^2					
C_6H_5	n-C_3H_7	9·5	16·7	1·5	1·63	21·7
			25·0	1·5	1·75	21·7
			50·0	1·5	1·50	21·7
		11·0	50·0	1·5	1·47	21·7
			50·0	5·0	2·10	72·3
			50·0	11·5	2·24	166·0
			50·0	23·0	2·45	332·0
			50·0	50·0	2·57	723·0
$C_6H_5CH_2$	n-C_3H_7	12·0	16·7	1·5	3·64	12·0
			25·0	1·5	2·66	12·0
			50·0	1·5	2·67	12·0
			50·0	5·0	5·06	37·7
			50·0	7·5	5·50	56·0
$C_6H_5CH_2$	CH_2COOH	12·0	25·0	2·5	7·13	18·9
			25·0	5·0	6·10	37·7
			50·0	2·5	7·22	18·9

[a] Measured by method C_2 at the wavelengths quoted in Table I; [b] calculated according to equation $k' = k'_{OH} + k'_{HS}$, where $k'_{OH} = k_{OH}c_{OH}$, and $k'_{HS} = k_{HS}c_{HS^-}$. The value $k_{OH} = 0{\cdot}119 M^{-1} . s^{-1}$ from ref.[3], $k_{HS} = 13{\cdot}18 M^{-1} s^{-1}$ is the rate constant of the reaction of corresponding ITC with sulfide according to ref.[2].

direct spectrophotometric determination of the relationship between the concentration of EDTC or DTC in the reaction mixture and time was made difficult. For this reason, we used the extraction procedure described in Experimental. In the case of the S-propylester of N-benzyl dithiocarbamic acid for which it was possible to measure spectrophotometrically the change in its concentration even without extraction we found out that the extraction itself did not affect the accuracy of the determination of k_{obs} (Table II).

Although the pK_I constants found have been obtained by different methods, their comparison for substance *VII* (Table I) shows practically full agreement which proves the reliability of the methods used. As mentioned, though the values E_t obtained by the method A are charged with the error due to a small percent decomposition at the moment t of reading extinction, we obtain the values of pK_I identical with the values obtained by the method C_2 provided the measured values were extrapolated to the time moment t_0 (the values E_0) by using the rate constants of EDTC decomposition (Table I, substances *II* and *VI*).

In agreement with other studies[26,27], the measurements of the UV absorption spectra of EDTC (Fig. 1*a* and 1*b*) confirmed the existence of the dissociated form *Ib* so as it ensued from our kinetic measurements. It follows from the relationship between the k_{obs} of EDTC decomposition and variables c_{H^+} and k_{obs} according to Eq. (*2*) and (*3*) that simple dissociation involving a transfer of one proton took place (Fig. 3*b*). Equal conclusion follows from the study of the relationship between E_{obs} and variables c_{H^+} and E_{obs} according to Eq. (*1*) (Fig. 2*b*). The values of the correlation coefficients indicate a high significance of these relationships (Table I). The effect of substituents R^1 and R^2 on the value of pK_I may be appreciated from the pK_a values of corresponding R^1NH_2 and R^2SH. The high differences in the pK_a values of R^1NH_2 also manifest themselves in considerable differences in the pK_I values of the substances studied. The appreciation of the effect of a substituent R^2 is limited by the close pK_a values of corresponding aliphatic thiols.

By using $R^1NHCSSR^2$ and buffer solutions with such values of pH at which these substances decayed, we succeeded in the presence of a sufficient excess of other thiol R^3SH to prepare EDTC of the structure $R^1NHCSSR^3$.

We consider the problem studied to be profitable not only from a purely theoretical point of view but also from the viewpoint of special problems, such as restoring of the catalytic activity of the SH enzymes inhibited by the reaction ITC, utilization of macromolecular polyisothiocyanates for the preparation of enzymes bound by the reaction with NH_2 groups and isolation of low-molecular and polymeric compounds containing mercapto group.

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