

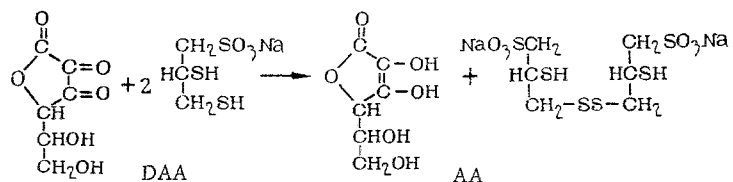
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REACTION OF DEHYDROASCORBIC ACID WITH UNITHIOL AND SOME
OF ITS KINETIC REGULARITIES

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Many compounds containing the thio group are proposed as ascorbic acid (AA) stabilizers in prepared medicinal forms: cysteine, thiourea, methylthiouracil, glutathione, etc. [1, 2]. Recently it was shown that introduction of sodium 2,3-dimercaptopropanesulfonate (unithiol) into injection solutions of AA strongly retards the development of coloration of the latter that appears upon lengthy storing of the ampuls [3, 4]. Darkening of the solution is caused by impurities of polymeric-type materials, which are formed upon oxidative decomposition of AA. In all probability the mechanism of stabilization by unithiol is based, on one hand, on its extraordinarily easy capability for oxidation [5], which leads to bonding of available oxygen in the ampul, and on the other hand, on the property of unithiol to form complexes with heavy metal ions [6], catalyzing decomposition of AA. As we have shown, it is also necessary to consider the ability of unithiol to reduce reversibly and easily the primary oxidation product of AA, dehydroascorbic acid (DAA). This paper is devoted to the study of reaction of DAA with unithiol as a result of which AA is formed.



Reaction of DAA with Unithiol. The investigation of the reaction of DAA with unithiol, not described earlier in the literature, showed that unithiol, like other sulfhydryl compounds [7, 8], reduces DAA to AA at a fast rate and quantitatively. We demonstrated formation of AA in the reaction process using UV spectroscopy and thin-layer chromatography.

Dilution of the reaction mixture (equimolar amounts of DAA and unithiol, pH 5.6) with buffer solutions of pH 0.7 (hydrochloride) and pH 6.5 (phosphate*) leads to formation of solutions having absorption maxima in the UV region at 244 and 265 nm, respectively. According to literature data, the first maximum corresponds to nondissociated AA (λ_{max} 244 nm, ϵ 10,000 [9], $\text{pK}_1 \approx 4.2$ [10]); the second corresponds to the AA monoanion (λ_{max} 265 nm, ϵ 14,300 [11], $\text{pK}_2 \approx 11.7$ [12]). Spectra of AA were taken for comparison under these same conditions. The ratio $\epsilon_{265}:\epsilon_{244}$ coincides well for both the reaction mixture and for individual AA (Table 1).

Formation of AA in the reaction mixture was also confirmed by data of thin-layer chromatography on silica gel in the systems: acetic acid-acetone-methanol-benzene (7.5:7.5:25:60) and 90% alcohol. A solution of o-phenylenediamine in acetic acid or alcohol was used as the developer.

*A stabilizer, sodium ethylenediaminetetraacetate, was added in an amount of 0.02% to the phosphate buffer solution.

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TABLE 1. Spectral Characteristics of the Reaction Mixture and AA Solutions at Different pH Values

Investigated solution	pH	λ_{\max}	ϵ	$\epsilon_{244}/\epsilon_{265}$
Reaction mixture	6,5	265	13 300	1,43
	0,7	244	9 300	
AA	6,5	265	13 750	1,42
	0,7	244	9 700	

TABLE 2. Limiting Degree of Transformation of DAA in Reaction with Unithiol at 10° (pH 5.6)

Initial concentration (mole/liter · 10 ³)		% transformation after 1.5 h
DAA	unithiol	
2,40	2,4	94,0
2,40	4,8	92,6
2,20	22,0	92,1
0,94	21,0	93,6
0,89	71,0	91,2

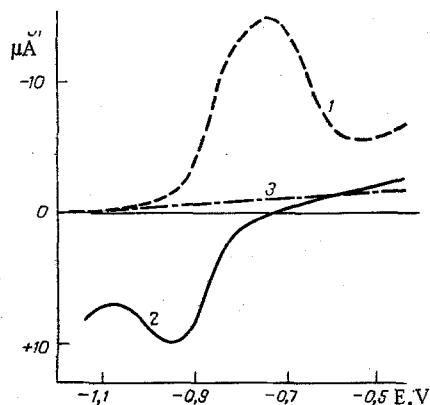


Fig. 1

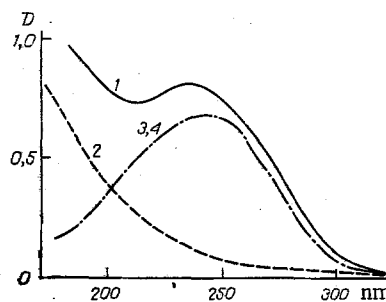


Fig. 2

Fig. 1. Polarographic curves of unithiol (1) and a mixture of DAA with unithiol (2) in acetate buffer solution (pH 5.6) after their dilution with base electrolyte (3).

Fig. 2. UV spectra after dilution with hydrochloride buffer solution of the reaction mixture of DAA + unithiol (1; DAA concentration $6.4 \cdot 10^{-5}$ mole/liter, unithiol concentration $6.5 \cdot 10^{-4}$ mole/liter), unithiol (2; concentration $6.4 \cdot 10^{-4}$ mole/liter), AA formed in reaction (3; as the difference of spectra 1 and 2), and AA (4; concentration $6.4 \cdot 10^{-5}$ mole/liter).

We did not identify the transformation products of unithiol; however, it was shown that during the reaction a new compound formed with a polarographic curve different from that of the initial material. ($\epsilon_{\max} \approx -0.8V$) (Fig. 1).

Decomposition products of unithiol virtually do not affect the UV spectrum of the reaction mixture (Fig. 2). Spectral curve (curve 3), obtained by graphic subtraction of absorption of unithiol (curve 2) from the spectrum of the reaction mixture (curve 1), coincides well with the known spectrum of AA (curve 4). Therefore, control of the course of the reaction of DAA with unithiol was achieved from the change in concentration of the formed AA.* For this purpose samples of the reaction mixture were diluted with hydrochloric buffer solution and the optical density was determined at λ 244 nm. The fraction of DAA (x) entering into reaction was found from the equation:

$$x = \frac{\epsilon_t}{\epsilon_{AK}}$$

*Contribution of absorption of DAA is quite small in the region of wavelengths of 240-270 nm; at λ 244 nm, $\epsilon = 94$ [9].

TABLE 3. Effective First-Order Constants (k_1) of Reaction of Unithiol with DAA in Acetate Buffer (pH 5.6) at 10°

Experiment No.	Initial concentrations (mole/liter)		$k_1 \cdot 10^4$ sec ⁻¹
	[DAA] · 10 ³	[unithiol] · 10 ²	
1	0,92	1,11	7,42
2	0,56	1,11	7,96
3	1,20	1,11	7,25
4	0,63	0,72	7,42
5	0,63	1,36	8,13
6	0,63	1,52	6,90
7	0,63	2,10	8,25
8	0,71	4,51	7,64
9	0,63	7,00	8,28

TABLE 4. Rate Constants (k_1 and k_2) of Reaction of DAA with Unithiol in Acetate Buffer Solution at 10°

pH	[OH] · 10 ¹⁰	$k_1 \cdot 10^4$ sec ⁻¹	$k_2 \cdot 10^{-5}$
4,38	0,702	0,45	6,41
4,70	1,466	1,21	8,25
4,92	2,432	2,00	8,22
5,10	3,681	2,83	7,69
5,60	11,640	7,68	6,60

Note. DAA concentration 8.4 · 10⁻⁴; unithiol concentration 2.1 · 10⁻² mole/liter.

where ϵ_t is the observable extinction coefficient of AA at moment of time t ; ϵ_{AA} is the molar extinction coefficient of AA in hydrochloric buffer solution at λ 244 nm, amounting to 9800.

The degree of transformation of DAA to AA at the end of reaction amounted to 91-94% (Table 2).

Order of Reaction with Respect to DAA and Unithiol. During the kinetic investigation we established the order of reaction with respect to each component: DAA and unithiol. Order of reaction with respect to DAA was established in experiments in which various initial DAA concentrations were used with a constant content of unithiol taken in excess (not less than tenfold) and a constant pH value. Values of effective rate constant k_1 calculated by the equation:

$$k_1 = \frac{2.303}{t} \log \frac{1}{1-x}, \quad (1)$$

for an assumed first-order reaction, differ from one another by not more than 10% (data obtained at a degree of transformation of from 10 to 85% were used in calculations).* Kinetic curve (1) and its semilogarithmic anamorphosis (2) are presented in Fig. 3 as an illustration. Points of (2) lie satisfactorily on a straight line. The first order of reaction in DAA is also confirmed by the fact that k_1 in experiments with the same initial unithiol concentration and different initial DAA concentrations coincide well† (Table 3, experiment Nos. 1-3).

Treatment of experimental results (Nos. 4-9, see Table 3), in which a constant initial DAA concentration and different unithiol concentrations were used (0.7-7.0 · 10⁻² mole/liter) at a constant pH, showed that k_1 virtually does not depend on the unithiol concentration. This indicates zero order of reaction with respect to unithiol for the studied concentration range.

Thus, at a constant pH the reaction goes by a first-order reaction law:

$$-\frac{d[\text{DAA}]}{dt} = k_1[\text{DAA}]. \quad (2)$$

Dependence of Rate of Reduction of DAA to AA on Medium pH. The effect of acidity of the medium on reaction rate constant k_1 was studied under conditions of identical initial concentrations of the other reaction components: unithiol and DAA. It follows from the dependence of k_1 on pH of the medium presented in Table 4 that the effective rate constant decreases with an increase in $[\text{H}^+]$, i.e., it increases with increasing $[\text{OH}^-]$. The order

*Since the reaction proceeds quite rapidly (the reaction is over in approximately 1 h at pH 5.6 and 10°) control was achieved by removal of sample; the study of its kinetics was carried out beginning with a degree of $\sim 10\%$.

† The restriction of initial DAA concentrations is associated with its low solubility in water.

TABLE 5. Dependence of Reaction Rate Constant on Temperature

t°	$K_W \cdot 10^{14}$ [12]	$k_1 \cdot 10^3 \text{ sec}^{-1}$	$k_2 \cdot 10^{-5}$ liter · mole ⁻¹ · sec ⁻¹
10	0,291	0,768	6,597
15	0,450	1,480	8,245
20	0,681	2,583	9,510
25	1,000	4,866	11,300

Note. DAA concentration $8.3 \cdot 10^{-4}$;
unithiol $2.5 \cdot 10^{-2}$ mole/liter; pH 5.6.

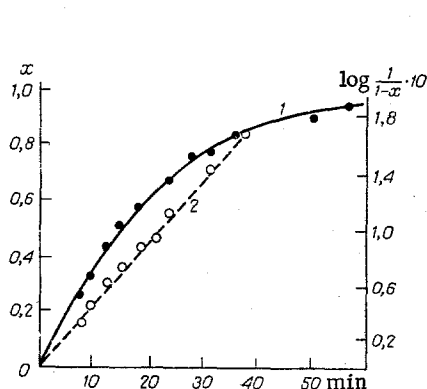


Fig. 3

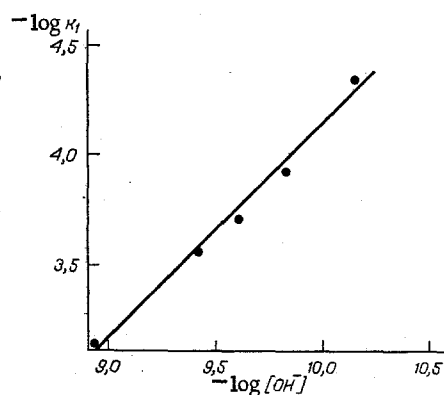


Fig. 4

Fig. 3. Kinetic curve (1) and its semilogarithmic anamorphosis (2) of the reaction of DAA with unithiol in acetate buffer solution (pH 5.6) at 10° (DAA concentration $5.6 \cdot 10^{-4}$ mole/liter, unithiol $1.1 \cdot 10^{-2}$ mole/liter).

Fig. 4. Dependence of $\log k_1$ on $[\text{OH}^-]$ for the reaction of DAA with unithiol.

in OH^- was determined from the dependence of $\log k_1$ on $\log [\text{OH}^-]$ at 10° . As is seen from Fig. 4, experimental points lie well on a straight line with a tangent of the angle of inclination of ≈ 1 , which corresponds to first order in OH^- . From this it follows that $k_1 = k_2[\text{OH}^-]$, where k_2 is the real second-order rate constant of reaction of DAA with unithiol (first order in DAA and OH^- , respectively). In such a case Eq. (2) takes the form:

$$-\frac{d[\text{DAA}]}{dt} = k_2[\text{DAA}][\text{OH}^-]. \quad (3)$$

Since the OH^- concentration is constant and in dilute solutions is equal to

$$[\text{OH}^-] = \frac{K_W}{[\text{H}^+]},$$

where K_W is the ionic product of water (Table 5), the value of the second-order reaction rate constant can be found. As is seen from Table 4, the agreement of the obtained value of k_2 is completely satisfactory.

Effect of Temperature. Dependence of rate constant k_2 on temperature in the range of from 10 to 25° obeys the Arrhenius equation (see Table 5, Fig. 5). The values of activation energy calculated by the method of least squares is equal to $5.893 \pm 0.316 \text{ kcal} \cdot \text{mole}^{-1}$. The preexponential term of the reaction amounts to $10^{10.4} \text{ liter} \cdot \text{mole}^{-1} \cdot \text{sec}^{-1}$.

Mechanism of the Reaction. Results of the study of kinetics of the reaction of quantitative reduction of DAA with unithiol to AA (first order of reaction in DAA, independence

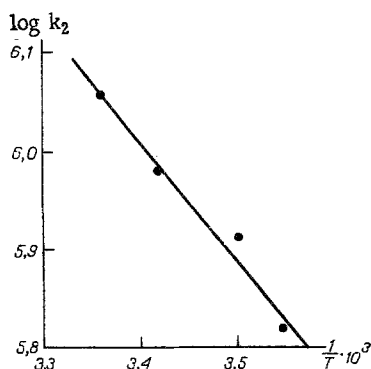
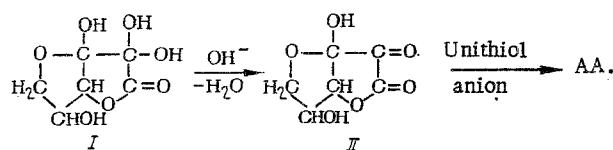


Fig. 5. Arrhenius dependence for the reaction of DAA with unithiol.

of reaction rate on unithiol concentration, inversely proportional dependence of reaction rate on hydrogen ion concentration) makes it possible to carry out an analogy with known thiol oxidation reactions in aqueous solution to disulfides under the effect of oxygen, hydrogen peroxide, and sodium persulfate [13]. It is proposed that in these reactions the reducing agent is thiolate anion [14]. Since the concentration of reducing agent does not enter into the expression found by us for the reaction rate (see Eq. (3)) the rate-determining step, in analogy with the transformations described above, is formation of an active intermediate compound from oxidizing agent (DAA) under the effect of OH⁻ ion. Such a compound can be considered an electrochemically active form of DAA having, according to data of [15], structure (II), which is in equilibrium with the inactive hydrated form (I) [16]. The constant k₂ obtained by us determines the rate of transformation of inactive DAA (I) to active (II) by the following scheme:



Electrochemically active form (II) is rapidly reduced further to AA with unithiol anion.* Intermediate formation of a complex is possible at this stage, in analogy with the described reaction product of DAA with glutathione [18] or an adduct as, for example, during oxidation of mercaptans with iodine [19] or dimethyl sulfoxide [20]. Decomposition of the latter leads to formation of AA and the corresponding oxidation product. Disulfide or tetrasulfide can be considered the most probable oxidation products of unithiol.

EXPERIMENTAL

Crystalline DAA (dimer) was obtained in a yield of 93% by oxidation of AA in methanol with air oxygen in the presence of palladium catalyst [21], bp 223-225° (from nitromethane).

Unithiol and medicinal AA satisfied all requirements of pharmacopoeia [22].

Reagents used in the research were "kh. ch." ["chemically pure"] and "ch. d. a." ["analytic"] grades. Acetic acid and sodium acetate trihydrate were used to prepare acetate buffer solutions. Concentration of initial solutions was 0.2 mole/liter. The phosphate buffer solution (0.028 mole/liter of Na₂HPO₄ and 0.042 mole/liter of KH₂PO₄) was prepared from salts recrystallized from water and dried at 110° (KP₂PO₄) or over calcium chloride (Na₂HPO₄).

Ampuls of fixanal with 0.1 N hydrochloric acid were used to prepare hydrochloride buffer solution (0.4 N hydrochloric acid, 0.4 N potassium chloride solution).

Spectrophotometry was carried out on a Specord UV-VIS instrument using square 1-cm cells.

Polarograms were taken on an LP-7 polarograph in a cell thermostated at 25° with a resistance of 500 Ω. A dropping mercury electrode was used as the cathode. Capillary

*Unithiol (sodium 2,3-dimercaptopropanesulfanate) is the salt of a strong sulfo acid; pK₂ and pK₃ of unithiol, corresponding to dissociation of the SH groups, are equal to 8.75 and 11.17, respectively [17].

characteristics: m 1.3 mg/sec, τ 4 sec. The anode was a normal calomel electrode. A solution containing 0.2 mole/liter of potassium hydroxide and 0.2 mole/liter of sodium sulfite served as the base electrolyte. Directly before use the solution was freed from traces of oxygen by flushing with nitrogen (15 min).

Experimental Method. A DAA solution ($0.5-1.2 \cdot 10^{-3}$ mole/liter) was thermostated for 20 min at 10° , simultaneously saturating with dry nitrogen purified from impurities, then poured into a measuring flask containing a weighed amount of unithiol, and mixed rapidly to complete solution of the latter. The obtained reaction mixture with an initial unithiol concentration of $0.7-7.0 \cdot 10^{-2}$ mole/liter was placed in a U-shaped cell thermostated at $10 \pm 0.5^\circ$, connected to a reflux condenser, and having an opening for sample removal. A stream of nitrogen was passed through the cell. Samples removed periodically from the reaction mixture were diluted with hydrochloric buffer solution, and the optical density (D) was measured at a wavelength (λ) of 244 nm (interval 0.15-0.95). A solution of pure unithiol maintained under the reaction conditions and diluted in the same ratio as the reaction mixture was placed in the reference cell. Samples of the latter were removed simultaneously with samples of the reaction mixture. The reaction was considered over when D changed by not more than ± 0.01 units per h.

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