results obtained in this work for cysteine–BSSB differ by a factor of about two from the values expected on the basis of probability and are in agreement with this conclusion.

Despite the fact that the constants are by no means unusual, the behavior of cysteine–BSSB is different from that of systems previously studied, and this serves to point out the important role which may be played by the buffer medium. At pH 7.0, for instance, BSH is almost completely ionized, while RSH is not. As a result, BSSB is almost completely reduced by an equivalent amount of RSH. Qualitative observations of similar import had been made by Eldjarn and Pihl, who reported that cystine was not reduced at pH 7.4 by aromatic mercaptans. It is important to realize this is not due to significant differences in the stabilities of the disulfide bonds involved, at least in the case of BSSB and RSSR, but to the fact that the acid–base reactions taking place with the buffer components provide the driving force for the reaction producing BS^-.

The case of BSH is of course an extreme one. With aliphatic mercaptans, little or no ionization will take place below pH 7, and hence there will be no effect on the interchange reaction. Above pH 7, *e.g.*, in the work of Eldjarn and Pihl that was conducted at pH 7.4, ionizations would certainly be appreciable. Their effect on the point of equilibrium is difficult to evaluate, however, since this depends on the difference between the ionization characteristics of the reactants and products, which are not known for the most part.

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Mercaptan–Disulfide Interchange Reactions. IV. Cysteine and Related Compounds with 3,5-Diimino-1,2,4-dithiazoline[1]

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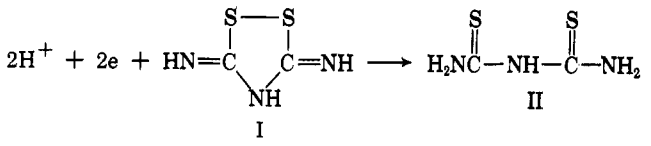
The reaction of 3,5-diimino-1,2,4-dithiazoline (DS_2) with cysteine, glutathione, and 2-aminoethanethiol is first order in DS_2 and second order in mercaptan; the specific rate constants are 9.4, 6.9, and 17.0 × 10^3 moles^2 l.$^{-2}$ min.$^{-1}$, respectively, at pH 4.60 and 30°. At pH 4.6–3.5, the rate is approximately proportional to the reciprocal of hydrogen ion activity. A mechanism is proposed.

This paper is part of a series[2] dealing with the interaction of disulfide and mercapto compounds, particularly those of importance in biochemistry. 3,5-Diimino-1,2,4-dithiazoline (DS_2, I) is a cyclic disulfide of unusual constitution, and a study of its reaction with cysteine and some cysteine derivatives was undertaken in the hope of finding some novel and useful results. DS_2 and dithiobiuret (II) possess the important property of forming a thermodynamically reversible half-cell (E_0 = +0.251 v. at pH 0).[3] The potential of other mercaptan–disulfide systems, which do not give reversible half-cells and are still to some extent uncertain,[4]



I II

might be determined if an equilibrium between them and DS_2–dithiobiuret could be established and measured.

This possibility has not yet been realized, principally for the reason that the reaction is very slow at the pH and concentrations that might otherwise be suitable. The present paper reports measurements of the reaction rates, and some deductions concerning the reaction mechanism.

Experimental

Materials.—Dithiobiuret, from the American Cyanamid Co., New York, N. Y., was recrystallized three times from hot 0.01 *M* hydrochloric acid, washed with ethanol, and dried *in vacuo*. 3,5-Diimino-1,2,4-dithiazoline hydrochloride was prepared as described by Preisler and Bateman.[3] 2-Aminoethanethiol (β-mercaptoethylamine), from Evans Chemical Co., New York 17, N. Y., was recrystallized from methanol and dried *in vacuo*. L-Cysteine hydrochloride hydrate, from California Corporation for Biochemical Research, Los Angeles 63, Calif., and glutathione, from Schwarz Laboratories, Mt. Vernon, N. Y., were used as obtained. All other chemicals were of reagent grade. The water used in the preparation of all solutions was distilled, passed through a column of Amberlite MB-1 ion-exchange resin, boiled for 20–25 min., cooled with a stream of nitrogen passing through, and stored under nitrogen for no longer than 2 days.

The buffers of pH 4.6–3.8 contained acetic acid–sodium acetate of total concentration 0.1 *M*; the buffers of pH 3.7 – 3.5 were prepared similarly from chloroacetic acid.

Apparatus.—Spectral measurements were done with a Beckman DU spectrophotometer, in 1-cm. silica cells. Measurements of pH were done with a Beckman Model GS pH meter, standardized with commercial buffers.

Kinetic Measurements.—Solutions of DS_2 and mercaptan were prepared in air-free buffer shortly before the measurements; in many but not all cases, the solutions were also made 10^{-3} *M* in ethylenedinitrilotetraacetic acid. Appropriate amounts were mixed to give about 5 × 10^{-5} *M* DS_2 and the desired mercaptan ratio, and an aliquot portion was transferred to a spectrophotometer cell. The absorbance at 246 and 282 mμ was then measured at intervals, against a blank solution containing mercaptan and buffer. The temperature was maintained at 30 ± 0.5°

(1) This work was supported by Contract AT(11-1)-71, No. 8, from the Division of Biology and Medicine, U. S. Atomic Energy Commission.

(2) See preceding papers: *J. Org. Chem.*, **29**, 1480, 1484 (1964).

(3) P. W. Preisler and M. M. Bateman, *J. Am. Chem. Soc.*, **69**, 2632 (1947).

(4) W. M. Clark, "Oxidation-Reduction Potentials of Organic Systems," Williams and Wilkins, Baltimore, Md., 1960, pp. 471–487.

with the aid of an attachment which has been described elsewhere.[5] Whenever it was desired to continue the measurements for longer than 15 min., the cell contents were renewed from the stock reaction mixture, which was kept in a thermostat under nitrogen.

Results

DS_2 is a base with ionization constants $10^{-7.4}$ and *ca.* 10.[3] It, therefore, exists as the cation $(DS_2H)^+$ in the pH range 4.6–3.5 employed in the experiments. $(DS_2H)^+$ has an absorption maximum at 246 mμ; dithiobiuret has a maximum at 282 mμ.[6] The molar absorbancies at these wave lengths are as follows.

	246 mμ	282 mμ
$(DS_2H)^+$	16,540	6,010
Dithiobiuret	4,100	19,630

When $(DS_2H)^+$ was mixed with excess cysteine or other mercaptan (RSH) in buffer, the absorbance at 246 mμ decreased and that at 282 mμ increased. If $(DS_2H)^+$ and dithiobiuret are the only absorbing species present, one can write for the absorbance at 246 or 282 mμ

$$A = A_{SS}[(DS_2H)^+] + A_{SH}\{[(DS_2H)^+]_0 - [(DS_2H)^+]\} \quad (1)$$

where A_{SS} and A_{SH} are the respective molar absorbancies and $[(DS_2H)^+]_0$ is the initial concentration. Rearrangement of the equation gives

$$[(DS_2H)^+] = \{A - A_{SH}[(DS_2H)^+]_0\}/(A_{SS} - A_{SH}) \quad (2)$$

Rate measurements were conducted in the presence of a large excess of mercaptan, the molar ratio [RSH]/$[(DS_2H)^+]$ being 20, 35, and 50. In these conditions it might be expected that the disappearance of $(DS_2H)^+$ would follow the first-order law, *i.e.*

$$\log [(DS_2H)^+]/[(DS_2H)^+]_0 = -kt/2.303 \quad (3)$$

so that a plot of $-\log \{A - A_{SH}[(DS_2H)^+]_0\}$ against t would be a straight line of slope $k/2.303$. This was in fact found experimentally; a representative set of data is shown in Fig. 1.

Table I summarizes the results ($= k'$) obtained by dividing the pseudo-first-order rate constants obtained at various mercaptan ratios by the mercaptan concentration squared. It can be seen that the results are constant for any given mercaptan, indicating that the rate is directly proportional to $[RSH]^2$. Ethylenedinitrilotetraacetic acid (EDTA) was added in some of the experiments, because this substance has been found effective in retarding autoxidation; however, it did not have an appreciable effect.

Table II summarizes the results obtained at 50:1 cysteine–$(DS_2H)^+$ ratio and pH values from 4.6 to 3.5. Measurements were not made at higher pH because DS_2 is reportedly unstable above pH 5,[3] while the lower limit was determined by the length of time required. The range covers a tenfold increase in hydrogen ion activity, and the rate decreases somewhat more rapidly than if it were inversely proportional to this quantity.

(5) H. J. Martin and G. Gorin, *Anal. Chem.*, **32**, 892 (1960).

(6) G. Baudo and G. Gorin, Abstracts, 137th National Meeting of the American Chemical Society, Cleveland, Ohio, April, 1960, p. 28-C. The assistance of Mr. Baudo in some of the early experiments is acknowledged with thanks.

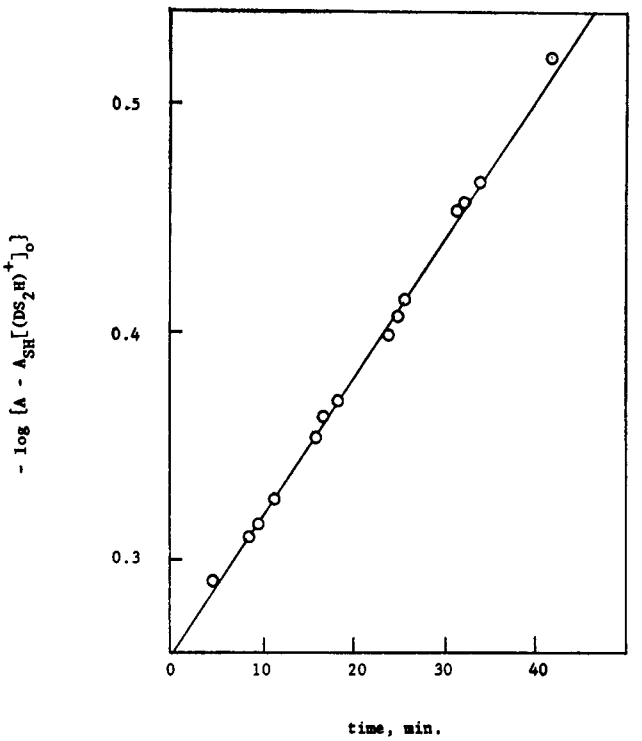


Fig. 1.—First-order rate equation plot for reaction of cysteine at 30°, pH 4.2. Initial concentrations are $[DS_2] = 4.54 \times 10^{-5}$, [cysteine] $= 2.26 \times 10^{-3}$ *M*.

TABLE I

THIRD-ORDER RATE CONSTANTS FOR REACTION OF $(DS_2H)^+$ WITH MERCAPTANS[a]

Mercaptan	Initial RSH-$(DS_2H)^+$ ratio 20:1	35:1	50:1	Mean
Cysteine	9.53[b]	8.75	9.83	
	8.89	9.12	10.22	9.4 ± 0.5
		9.21[b]	8.96	
			10.13[b]	
2-Aminoethanethiol	17.2[b]	17.8[b]	16.73[b]	
	17.3[b]	16.9[b]	16.9	17.0 ± 0.3
		16.5		
Glutathione	7.02[b]	6.84[b]	6.68[b]	
	6.91	7.00	6.93	6.9 ± 0.9

[a] pH 4.60, 30°; units of k' are 10^3 l.^{2} mole^{-2} min.$^{-1}$. [b] Denotes ethylenedinitrilotetraacetic acid added.

TABLE II

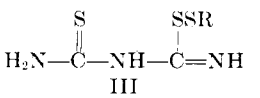
pH DEPENDENCE OF RATE CONSTANTS[a]

pH	$10^{-3}\,k'$, l.^{2} mole^{-2} min.$^{-1}$	$k'[H^+]$,[b] l. mole^{-1} min.$^{-1}$
4.60	9.40	0.235
4.30	4.14	0.211
4.00	1.75	0.175
3.70	0.96	0.192
3.50	0.58	0.186

[a] [Cysteine–$(DS_2H)^+$] = 50, 30°. [b] $[H^+] = 10^{-pH}$.

Discussion

The following mechanism is approximately in accord with the results reported in the preceding section. It is postulated that $(DS_2H)^+$ reacts with mercaptan to form the mixed disulfide (III, dithiobiuret = DTB).



III

$$(DS_2H)^+ + RSH \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} III + H^+ \quad (a)$$

$$III + RSH \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} (DTB) + RSSR \quad (b)$$

It is further postulated that III is an unstable intermediate, whose concentration remains small and nearly constant throughout the reaction, so that one can write to a good approximation

$$d[(III)]/dt = k_1[(DS_2H)^+][RSH] - k_{-1}[(III)][H^+] - k_2[(III)][RSH] \cong 0 \quad (4)$$

whence

$$[(III)] = \{k_1[(DS_2H)^+][RSH]\}/\{k_{-1}[H^+] + k_2[RSH]\} \quad (5)$$

Finally, it is postulated that $k_{-1}[H^+] >> k_2[RSH]$. Since, in the conditions, the reversal of eq. b is negligible, one can write

$$-d[(DS_2H)^+/dt = d[(DTB)]dt = k_2[(III)][RSH] = \{k_1k_2[(DS_2H)^+][RSH]^2\}/k_{-1}[H^+] \quad (6)$$

In the presence of a large excess of RSH and at constant pH, one can equate

$$k_1k_2[RSH]^2/k_{-1}[H^+] = k \quad (7)$$

The resulting expression can be integrated to give eq. 3. The specific rate constants reported in Tables I and II are related in the following ways

$$k_1k_2/k_{-1} = k'[H^+] = k[H^+]/[RSH]^2 \quad (8)$$

(in view of the approximate nature of the analysis, the distinction between hydrogen ion concentration and activity is not considered).

In other mercaptan–disulfide reactions it has been found that the equilibrium constants[2] for the reactions corresponding to eq. a and b are not very different from unity, from which one can conclude that the rate constants are usually of the same order of magnitude. Also, convincing evidence has been produced that the mechanism involves the reaction of disulfide with RS^-.[7] The mechanism proposed above for $(DS_2H)^+$ implies two principal points of difference. First, it is postulated that the rate of reversal of eq. a is greater than the forward rate of b; this can be rationalized on the grounds that, in the former case, the participating groups are held in close proximity at all times, the reaction being an intramolecular one. Secondly, $(DS_2H)^+$ must be more susceptible to S–S bond fission than ordinary disulfides, since the former can be attacked by RSH and the latter only by the more strongly nucleophilic RS^-. It should be noted that, in the pH range in question, $[RS^-]$ is very small; *i.e.*, it is not implied that $(DS_2H)^+$ cannot react with RS^-, only that the reaction is not important in the conditions employed. As a matter of fact, a likely explanation for the fact that the rate of reaction increases somewhat more rapidly with pH than predicted by eq. 6–8 is that RS^- makes an appreciable contribution to the over-all reactions at the higher pH values. The reaction with RS^- cannot be the principal one, however, as this would lead to a dependence of the rate on $1/[H^+]^2$, which is clearly not the case.

A degree of uncertainty attaches to the above analysis, because the spectrum of the mixed disulfide is not known. Owing to this, one cannot be sure that the mixed disulfide would not be produced in appreciable amounts, its presence escaping detection by a fortuitous compensation of the absorbances. However, this is not likely. The mechanism proposed, though speculative, provides a reasonable explanation for the experimental findings.

(7) O. Foss in "Organic Sulfur Compounds," Vol. I, N. Kharasch, Ed., Pergamon Press, New York, N. Y., 1961, pp. 87–90.

The Solvolysis of 3-Hydroxyestr-5(10)-en-17-one Sulfonates

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Solvolyses of the sulfonates of 3α- and 3β-hydroxyestr-5(10)-en-17-one (**2a, 6a**) yield the cyclosteroids **4** and **8**, respectively. Other products of the solvolyses include the diene **11a** and the *inverted* alcohols. The mechanisms involved in the synthesis of these compounds are discussed.

i-Steroids, both in structural determination and the mechanism of formation, have been the basis of a fascinating story through several decades of organic chemistry.[1] Investigation in this area continues, in recent years concentrated largely on determination of the steric requirements necessary for the synthesis of these compounds[2] and on a more precise definition of the nonclassical cation intermediate in their formation.[3] Pertinent to both problems, the present work reports the results of experiments dealing with the C-3 sulfonates of $\Delta^{5(10)}$-steroids.[4] These compounds, containing both the participating double bond and the oxygen function in the *same* (unbridged) ring, represent a structural type whose solvolyses have not been investigated heretofore. Numerous examples exist of bridge 3-cyclohexenol sulfonate solvolyses.[5] How-

(1) L. F Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, p. 314; a recent list of *i*-steroid syntheses has been given by L. H. Knox, E. Velarde, S. Berger, D. Cuadriello, P. W. Landis, and A. D. Cross [*J. Am. Chem. Soc.*, **85**, 1851 (1963)].

(2) For leading references see the following: W. J. A. Vandenheuvel and E. S. Wallis, *J. Org. Chem.*, **27**, 1233 (1962); C. W. Shoppee and G. A. R. Johnston, *J. Chem. Soc.*, 3261 (1961); R. M. Moriarty and R. M. deSousa, *J. Org. Chem.*, **28**, 3072 (1963).

(3) (a) S. Winstein and E. M. Kosower, *J. Am. Chem. Soc.*, **81**, 4399 (1959) and references cited there; (b) G. H. Whitham, *Proc. Chem. Soc.*, 422 (1961).

(4) Portions of this work are included in U. S. Patents 2,944,067 (1960), 2,944,068 (1960), and 3,087,943 (1963). Work along similar lines has been recently reported by S. G. Levine, N. H. Eudy, and E. C. Farthing [*Tetrahedron Letters*, 1517 (1963)].

(5) J. A. Berson, "Molecular Rearrangements," P. DeMayo, Ed., Interscience Publishers, Inc., New York, N. Y., 1963, p. 192, and references contained there.