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The Alkaline Decomposition of Organic Disulfides. II. Alternative Pathways as Determined by Structure[1]

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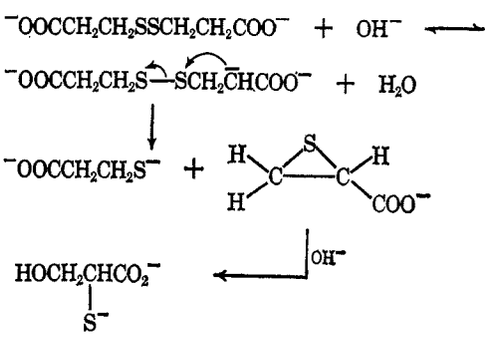
Determination of the relative speeds of decomposition, and of the products formed, for 17 disulfides bearing widely varying substituents in aqueous alkali (pH 9 to 14) at 35.2° leads to the following view, to which no exception has been observed. (1) The aqueous, alkaline decomposition of aliphatic disulfides is initiated either by direct nucleophilic attack of hydroxide ion on one of the sulfur atoms, or by proton abstraction from a carbon atom α or β to the sulfur atom. (2) Direct attack on sulfur is strongly inhibited by a negative charge and strongly accelerated by a positive charge on the disulfide. Dithiodicarboxylic acids, whose carboxylate anions inhibit direct attack, either do not decompose at all or appear to have their decompositions initiated by proton abstraction. Subsequent steps and final products are determined by other structural features of the individual disulfides.

The extensive literature on the alkaline decomposition of organic disulfides has recently been reviewed in detail by Danehy,[2] and more briefly by Danehy and Kreuz.[3] While Schöberl[4] originally proposed direct hydrolytic attack as the initiating step, more recently, elimination schemes have been favored. Swan[5] has furnished some interesting data in support of the view that the aqueous, alkaline decomposition of cystine (IX) proceeds *via* a β elimination, and we[3] have interpreted our quantitative data for the decomposition of dithiodiacetic acid (II) and of 2,2′-dithiodipropionic acid (III) in terms of an α elimination. As we have continued to study the details of the alkaline decompositions of a variety of water-soluble disulfides, a pattern has emerged which, while it incorporates some of the ideas previously advanced, requires the rejection of other ideas in order to accommodate the experimental facts. The data appear consonant with the view that direct nucleophilic attack of the hydroxide ion on a sulfur atom initiates decomposition unless this event is inhibited by the presence of a negative charge on the disulfide. When direct attack does take place (under anaerobic conditions, of course) the major terminal products are thiol and sulfonic acid, in a molar ratio that approximates 5:1. When direct attack is inhibited the disulfide is either stable toward alkali, or decomposes in such a way as to suggest that proton abstraction from a carbon atom α or β to a sulfur atom is the initiating step.

SCHEME I



Discussion

It has already been reported[3] that 3,3′-dithiodipropionic acid (XI), even upon extensive decomposition in aqueous alkali, yields no hydrogen sulfide whatsoever: the disappearance of each mole of XI is accompanied by the appearance of 2 moles of thiol. Kreuz[6] has suggested the mechanism in Scheme I as a possible interpretation of the data.

Scaled-up experiments, designed for the isolation of the postulated 2-mercapto-3-hydroxypropionic acid, failed in their original purpose, but did yield the significant information that hydrogen sulfide begins to appear when the initial concentration of XI is increased to 0.004 *M*, and that the fraction of total sulfur which appears as hydrogen sulfide increases as the initial concentration of XI is increased beyond 0.004 *M* (Table I). It seems reasonable that increasing the

(1) Presented at the 151st National Meeting of the American Chemical Society, Pittsburgh, Pa., March 29, 1966. Based on the doctoral dissertation of W. E. Hunter.

(2) J. P. Danehy, "Chemistry of Organic Sulfur Compounds," Vol. II, N. Kharasch and C. Meyers, Ed., Pergamon Press Inc., New York, N. Y., 1966, Chapter 13.

(3) J. P. Danehy and J. A. Kreuz, *J. Am. Chem. Soc.*, **83**, 1109 (1961).

(4) A. Schöberl, *Ann.*, **507**, 111 (1933).

(5) J. M. Swan, *Nature*, **179**, 965 (1957); J. M. Swan and I. M. Stapleton, *Australian J. Chem.*, **13**, 416 (1960).

(6) J. A. Kreuz, Doctoral Dissertation, University of Notre Dame, 1959.

TABLE I

DECOMPOSITION OF 3,3′-DITHIODIPROPIONIC ACID (XI IN 1.68 *N* SODIUM HYDROXIDE AT 35.2°

Time, days	[RSSR], $M \times 10^4$	[RSH], $M \times 10^4$	[H_2S], $M \times 10^4$	% decompn	Sulfur balance, %
0	9.3	0.0	0.0	...	...
1	6.9	4.8	0.0	25.8	100
2	6.1	6.4	0.0	34.4	100
4	5.3	8.1	0.0	43.0	102
6	4.7	9.2	0.0	49.5	100
0	42.5	0.0	0.0	...	...
1	34.9	15.1	0.25	17.8	100
2	33.9	16.7	0.20	20.5	99
3	32.2	19.3	0.20	24.3	98
7	30.8	21.5	0.20	27.4	98
0	350.	0.0	0.0	...	...
1	290.	55.0	7.0	17.2	92
2	254.	102.0	11.0	28.1	89

initial concentration of XI would increase the likelihood of thiol–disulfide exchange reactions. Since a disulfide containing the postulated thiol in oxidized form should be as sensitive to alkali as III, it should decompose in normal sodium hydroxide almost as fast as formed. The following continuation of Scheme I, which was invoked to account for the appearance of hydrogen sulfide, suggests that hydroxypyruvic acid might also be formed. In order to investigate this possibility, 2,4-dinitrophenylhydrazine was added to acidified aliquots of an alkaline decomposition run of XI, after the precipitated XI had been removed by filtration, and an orange derivative, melting at 160–161°, containing 23.4% nitrogen, was obtained (see Table II).

TABLE II

DECOMPOSITION OF 3,3′-DITHIODIPROPIONIC ACID (XI) IN CONCENTRATED SOLUTION AT 35.2° IN 1.5 *N* SODIUM HYDROXIDE WITH ISOLATION OF DNPH DERIVATIVE

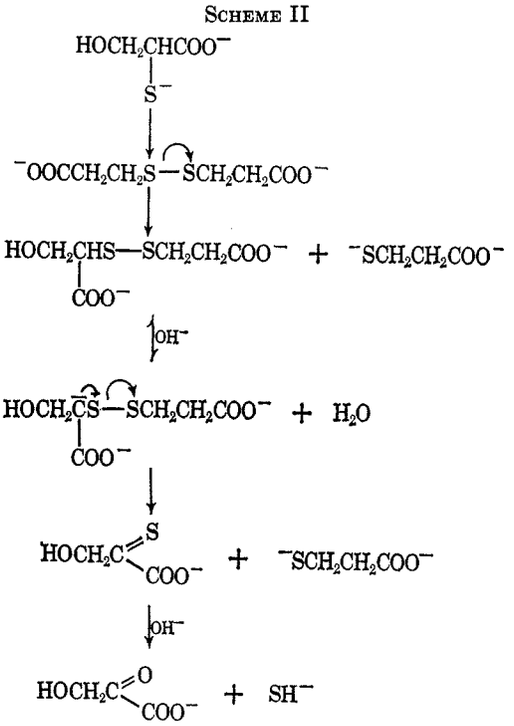
Time, days	[RSSR], *M*	[RSH], *M*	[H_2S], *M*	DNPH derivative, mg[a]	% decompn	Sulfur balance, %
0	0.5143	0.0	0.0	...	...	...
1.0	0.4509	0.0335	0.0145	19.5	12.4	92.5
2.0	0.4464	0.0626	0.0167	47.9	13.2	94.5
3.0	0.4423	0.0645	0.0152	34.9	14.0	93.7
8.0	0.3857	0.1062	0.0170	79.3	25.1	86.8

[a] Possibly 2,4-dinitrophenylhydrazone of hydroxypyruvic acid.

Sprinson and Chargaff[7] have shown that hydroxypyruvic acid (HPA) and 2,4-dinitrophenylhydrazine react to form an orange HPA 2,4-dinitrophenylhydrazone, melting at 162°, containing 19.8% nitrogen. They have also shown that HPA is rather unstable in aqueous alkali, in which it is transformed gradually to the corresponding enediol, and that acidified aliquots of an alkaline solution of HPA which has been stored for some time react with phenylhydrazine to give glyoxalosazone, pale yellow, melting at 169°. They have also oxidized the hypothetical enediol intermediate with iodine, acidified, and added phenylhydrazine to produce HPA phenylosazone, mp 222–223° dec, containing 19.9% nitrogen.

Our orange derivative is not the glyoxal 2,4-dinitrophenylosazone which might have been expected from the results of Sprinson and Chargaff, for Glasstone and Hickling[8] have reported that glyoxal 2,4-dinitrophenylosazone melts at 330°. On the basis of melting point, our derivative might be the 2,4-dinitrophenylhydrazone of HPA, but the nitrogen content is too high. On the basis of nitrogen content it might be the 2,4-dinitrophenylosazone of HPA, not previously reported, but the likelihood of the latter forming is small in view of the results of Sprinson and Chargaff. More work is needed to clarify this point. (See Scheme II.)

SCHEME II



Tables III and IV summarize the quantitative studies of the alkaline decomposition of 2,2′-dithiodiethanol (V) and of 2,2′-dithiodiethylamine (VI).[9] Neither increasing the disulfide concentration nor the alkaline concentration leads to any detectable trace of hydrogen sulfide. The studies on the decomposition of II, III, and XI predisposed us to an interpretation involving an elimination reaction, and, since the results in the earlier stages of decomposition of V and VI seem to indicate the appearance of 2 moles of thiol for each mole of disulfide which disappears, the episulfide mechanism seemed worthy of consideration; it would call for the production of equivalent amounts of 2-mercaptoethanol (or 2-mercaptoethylamine) and mercaptoacetaldehyde (plus 50% deamination in the case of VI). The further reaction of mercaptoacetaldehyde in alkali might account for the gradually decreasing sulfur balances actually observed.

Further study, however, forced abandonment of this position. Hesse and Jörder,[10] in attempting to prepare mercaptoacetaldehyde, obtained only the crystalline dimer, 2,5-dihydroxy-1,4-dithiane. Their claim that solutions of the dimer are in equilibrium with small

(7) D. B. Sprinson and E. Chargaff, *J. Biol. Chem.*, **164**, 417 (1946).

(8) S. Glasstone and A. Hickling, *J. Chem. Soc.*, 824 (1936). They call the compound "glyoxal 2,4-dinitrophenyl hydrazone," but it is clear from the empirical formula and analysis that they mean the osazone.

(9) In an earlier phase of this work Doris Smith, supported by National Science Foundation research grant G-10344, obtained consonant data which were reported at the 140th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1961, Abstracts, p 73Q.

(10) G. Hesse and I. Jörder, *Chem. Ber.*, **85**, 924 (1952).

TABLE III

DECOMPOSITION OF 2,2'-DITHIODIETHANOL (V) IN AQUEOUS SODIUM HYDROXIDE AT 35.2°[a]

[NaOH], N	Time, days	[RSSR], $M \times 10^4$	[RSH], $M \times 10^4$	% decompn	Sulfur balance, %
0.1025	0	10.8	0	...	...
	1.0	10.75	0.14	1.0	100
	4.2	10.68	0.26	2.0	100
	8.2	10.15	1.31	6.1	100
	19.2	8.14	5.33	24.7	100
	29.0	5.58	8.25	48.4	90
0.9728	0	11.9	0	...	...
	1	9.05	5.78	24.0	100
	2.3	6.73	6.63	43.0	85
	3.2	3.10	7.82	73.0	59
1.025	0	127	0	...	...
	0.125	119	18	6.3	100
	0.25	89	34	30.0	84
	0.42	79	51	37.8	83
	1.00	61	77	52.0	79
1.025	0	679	0	...	...
	0.042	644	50	5.2	99
	0.125	594	67	12.7	93
	0.25	505	140	25.7	85
	0.42	416	185	38.8	75

[a] No hydrogen sulfide observed at any time.

TABLE IV

DECOMPOSITION OF 2,2'-DITHIODIETHYLAMINE (VI) IN 1.10 N SODIUM HYDROXIDE AT 35.2°[a]

Time, hr	[RSSR], $M \times 10^3$	[RSH], $M \times 10^3$	[NH_3], $M \times 10^3$	% decompn	Sulfur balance, %
0	11.7	0	0	...	...
1.0	10.9	1.6	0	6.9	100
3.0	10.3	2.9	0	12.0	100
6.0	9.7	3.6	0	17.1	98
12.0	8.6	4.9	0	26.5	94
25.3	8.2	6.0	0.14	30.0	95
72.0	5.6	8.4	0.19	52.2	84
176.0	4.2	10.8	0.68	64.1	82

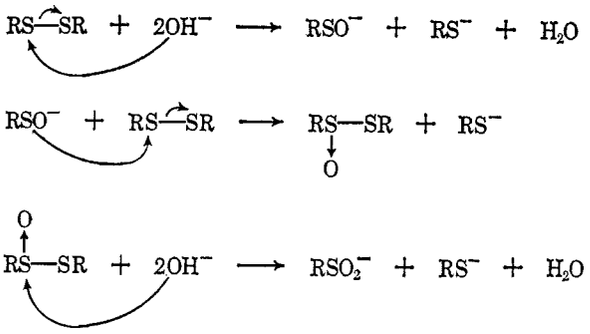
[a] No hydrogen sulfide detected at any time.

amounts of the monomer is supported by our observation that an aqueous solution of the dithiane, analyzed by the standard procedure with phosphotungstic acid, gives in 30 min a little less than 50% of the optical density that would be given by a normal thiol in 15 min; in 24 hr or less, full color development is attained. They also found, and we have confirmed, that the dithiane resinifies in aqueous alkali; but our aqueous, alkaline solutions of V and VI, even when extensively decomposed, are clear and only lightly colored (yellow). Moreover, work-ups of runs of V on a preparative scale have yielded as much as 80% of the decomposed disulfide in the form of the mercury mercaptide of 2-mercaptoethanol. Since the derivative obtained had a sharp melting point without recrystallization it seems that 2-mercaptoethanol is the only thiol produced in significant amount by the decomposition of V. Furthermore, the quantitative determination of the formation of ammonia from an alkaline solution of VI indicates that only 2.9% of the total nitrogen has appeared as ammonia while 64% of VI had decomposed.

The following experimental evidence has led to the reconsideration of the possibility of direct nucleophilic attack of the hydroxide ion on one of the sulfur atoms in a disulfide linkage. The corresponding sulfonic acids (isethionic acid from V, and taurine from VI) were isolated from preparative runs as their ammonium salts and identified by the melting point in one case and by thin layer chromatography with reference to authentic samples in both cases. In addition, the molar ratio of thiol to sulfonic acid was determined to be about 4:1 by titration of the latter for a preparative run of V which had been passed through a column of cation-exchange resin in the hydrogen ion form in order to remove sodium and hydroxide ions.

In the case of aromatic disulfides, it has long been known that the action of aqueous alkali produces a mixture of thiol and sulfinic acid.[11] None of the investigators cited has proposed any mechanistic interpretations, although Parker and Kharasch[12] have discussed some of the implications of the data. It seems reasonable to suggest that the sequence in Scheme III might be operative in the case of either aliphatic or aromatic disulfides.

SCHEME III



In general, aromatic sulfinic acids are more stable than aliphatic ones.[13] With the single exception of 3-sulfinoalanine (cysteine sulfinic acid, CSA), none of the aliphatic sulfinic acids of interest in connection with the present research problem has evre been reported. Lavine[14] has reported that CSA is an uncommonly stable sulfinic acid: it has appreciable resistance to oxidation by iodine; indeed, it oxidizes hydrogen iodide quantitatively to iodine. It is in the case of the alkaline decomposition of cystine (IX), then, that we might most likely expect to find a sulfinic acid among the decomposition products.

When alkaline solutions in which IX had decomposed extensively were passed through columns of cation-exchange resin in the hydrogen ion form, the acidic effluents, free of IX and of cysteine, were indeed found to contain CSA, but as determined quantitatively by Lavine's method[14] it amounts to only 20–25% of the total acidity as measured by titration with standard alkali.[15] The predominant acidic product is cysteic acid (*vide infra*).

The problem, under active investigation at the present time, is how sulfinate ion (probably, but not necessarily, present in each case), in the presence of strong alkali, thiol, and residual disulfide, is trans-

(11) R. Schiller and R. Otto, *Ber.*, **9**, 1637 (1876); E. Fromm and J. Wittmann, *ibid.*, **41**, 2266 (1908); E. Fromm, *ibid.*, **41**, 3403 (1908); S. Smiles and J. Stewart, *J. Chem. Soc.*, **119**, 1792 (1921); G. Pappalardo, *Gazz. Chim. Ital.*, **90**, 648 (1960); *Ann. Chim.* (Rome), **53**, 630 (1963).

(12) A. J. Parker and N. Kharasch, *Chem. Rev.*, **59**, 608 (1959).

(13) W. E. Truce and A. M. Murphy, *ibid.*, **48**, 69 (1951).

(14) T. F. Lavine, *J. Biol. Chem.*, **113**, 571, 583 (1936).

(15) Data provided by Dr. K. N. Parameswaran.

formed into sulfonate anion. The disproportionation of aromatic sulfinic acids, long known, has recently been reinvestigated.[16] In view of the facts, that disproportionation is very rapid in glacial acetic acid and is greatly retarded by the addition of water, and that sulfinate anions are more stable (at least, dry) than free sulfinic acids,[17] it is very unlikely that either of the pathways suggested by Allen or by Kice and co-workers could be operative in strong alkali. It does not appear that a study has yet been made of the behavior of sulfinate anions alone in strong alkali, in the absence of air. Kice and co-workers[18] have demonstrated that *p*-toluenesulfinic acid reacts with *p*-tolyldisulfide in acetic acid (0.5 *M* H_2O, 0.5 *M* H_2SO_4) at 70° to give the corresponding thiolsulfonate. Here, too, the medium is rather strongly acidic. Is it nevertheless possible that by a different mechanism the sulfinate ion might attack disulfide to give thiol and thiolsulfonate, and that the latter might undergo alkaline hydrolysis to furnish thiol and sulfonate? This view appears improbable in the light of Lavine's[14] work, for he reported that just the reverse of the first of these hypothetical reactions goes quantitatively in neutral, acidic, or alkaline media; *i.e.*, thiolsulfonate and thiol react to give disulfide and sulfinate.[19] He also reported that, in alkaline solution, thiolsulfonate is cleaved to yield only disulfide and sulfinate.

At present a complete mechanism for the aqueous, alkaline decompositon of many disulfides cannot be offered, since the formation of sulfonate as a terminal product cannot be explained. In those cases in which sulfonate is formed, however, the initial nucleophilic attack of hydroxide ion on one of the disulfide sulfurs is considered to be consistent with the evidence presented and with the relations developed below.

The question arises as to why the hydroxide ion seems to make a direct nucleophilic attack on sulfur in the cases of V and VI, but seems to abstract a proton in the case of XI. With II and III the case for α elimination has been stated in the first paper of this series, but here the likelihood of one alternative rather than the other is not so clear. It might be objected, in the case of XI, that the data presented do not rule out the possibility that some direct attack has taken place. That is true, but a number of other recently discovered facts are relevant to this question.

Neither dithiodiisobutyric acid (XVI) nor dithiodipivalic acid (XV) show any trace of decomposition after 15 days in 1.09 *N* NaOH. Structural features preclude an elimination reaction and failure to undergo direct attack is a matter of empirical observation. The speed of alkaline decomposition of dithiodiisovaleric acid (XII), however, almost exactly matches that of XI. Structurally, XII should be as susceptible to an elimination reaction as is XI. Of all the disulfides that decompose at all in aqueous alkali, 4,4′-dithiodibutyric acid (XIV) decomposes most slowly. In 0.103 *N* NaOH no decomposition could be detected in 28 days. In stronger alkali it decomposes about as fast as XI, but with distinct qualitative differences. In a preparative run in which the concentration of XIV was initially 0.145 *M*, no hydrogen sulfide was detected when XIV had decomposed to the extent of 60%, and 4-sulfobutyric acid was isolated and identified.

In view of the evidence it seems not unreasonable to suggest that a carboxylate anion sufficiently close to a sulfur atom in a disulfide inhibits direct attack on sulfur by hydroxide ion, presumably by mutual charge repulsion. Cecil and McPhee[20] have noted a similar relationship in the analogous case of nucleophilic attack of sulfite ions on disulfides. A limited study of 2,2′-dithiodiethanesulfonic acid (XIII) gives evidence consistent with this view: no decomposition in 0.109 *N* NaOH in 13 days, though it decomposes somewhat more rapidly than XI in stronger alkali. It is not known whether hydrogen sulfide may be a product of decomposition when the initial concentrtaion of XIII is substantially higher than 10^{-3} *M*. The structure would permit consideration of the episulfide mechanism.

More cogent, however, is the evidence obtained by eliminating the charge. Table V gives a direct comparison between the alkaline decompositions of XI and its diethyl ester (VIII). The initial rate of decomposition of the ester (VIII) must be at least three times as great as that of the corresponding acid (XI). Moreover, in 1.5 *N* NaOH the rate of hydrolysis of the ester, which transforms VIII into XI must be appreciable, so that the actual difference in the rates of disulfide decomposition may well be greater than that found experimentally. Even more striking is the difference between dithiodipivalic acid (XV), which is completely resistant to 1.09 *N* NaOH for at least 15 days, and its amide (VII), which is 94% decomposed in only 8 hr in 0.55 *N* NaOH at 35.2°.

TABLE V

DECOMPOSITION OF 3,3′-DITHIODIPROPIONIC ACID (XI) AND OF DIETHYL 3,3′-DITHIODIPROPIONATE (VIII) IN 1.5 *N* SODIUM HYDROXIDE AT 35.2°[a]

Compd	Time, hr	[RSSR], $M \times 10^3$	[RSH], $M \times 10^3$	% decompn	Sulfur balance, %
XI	0	12.1	0.0	...	...
	9.7	11.2	1.8	13.2	94
	23.0	10.7	2.8	17.1	94
VIII	0	12.1	0.0	...	...
	9.7	6.83	9.48	43.6	96
	23.0	4.90	10.8	59.5	85

[a] No hydrogen sulfide observed at any time.

The considerable evidence for the view that a negative charge on the disulfide, not too far removed from one of the sulfur atoms, inhibits direct nucleophilic attack on the latter by hydroxide ion simultaneously suggests the possibility that a positive charge on a disulfide might considerably accelerate direct attack of hydroxide ion on sulfur. Accordingly, 3,4-dithiahexane-1,6-bistrimethylammonium iodide (I) was synthesized and found to undergo 72% decomposition in only 1 hr in 0.106 *N* NaOH. The data in Table VI record the course of its decomposition in a carbonate–bicarbonate buffer at pH 9.37.

(16) P. Allen, Jr., and L. Reich, *J. Phys. Chem.*, **64**, 1928 (1960); J. L. Kice and K. W. Bowers, *J. Am. Chem. Soc.*, **84**, 605 (1962); J. L. Kice, D. C. Hampton, and A. Fitzgerald, *J. Org. Chem.*, **30**, 882 (1965).

(17) B. Lindberg, *Acta Chem. Scand.*, **17**, 377 (1963).

(18) J. L. Kice and K. W. Bowers, *J. Am. Chem. Soc.*, **84**, 2384 (1962); J. L. Kice and E. H. Morkved, *ibid.*, **86**, 2270 (1964).

(19) L. Field and co-workers [*J. Am. Chem. Soc.*, **83**, 4414 (1961)] have used this general method to synthesize a number of unsymmetrical disulfides, most of them aromatic.

(20) R. Cecil and J. R. McPhee, *Biochem. J.*, **60**, 496 (1955).

TABLE VI

DECOMPOSITION OF 3,4-DITHIAHEXANE-1,6-BISTRIMETHYLAMMONIUM IODIDE (I) IN CARBONATE–BICARBONATE BUFFER AT pH 9.37 AT 35.2° [a]

Time, days	[RSSR], $M \times 10^4$	[RSH], $M \times 10^4$	% decompn	Sulfur balance, %
0	10.3	0.0	...	...
1.0	10.0	0.56	3.0	100
2.0	9.25	1.60	10.0	98
3.0	8.72	2.68	15.4	98
5.4	6.15	4.77	40.0	83

[a] No hydrogen sulfide observed at any time.

The case of cystine (IX) continues to be of special interest for at least two reasons: (1) the large number of experimenal studies which have been made without a definitive accounting for IX in terms of identified products; (2) the fact, previously pointed out,[2,3] that cystinyl residues in proteins are readily transformed under moderately alkaline conditions into lanthionyl residues, although there are no authenticated cases of any organic disulfides, other than in proteins, being transformed into thio ethers by aqueous, alkaline treatment.[21]

The earlier work of Kreuz[6] established unequivocally that hydrogen sulfide is never a product of the anaerobic decomposition of IX at 35.2°, over a wide range of alkalinities and initial concentrations of IX. It is only recently, however, that we have been able to establish that cysteic acid ($CysSO_3H$) is a definite product of the reaction, and perhaps the only product other than cysteine (CysSH) and the small amount of CSA already noted. The qualitative presence, and the approximately quantitative determination, of cysteic acid has been established by ion-exchange column chromatography, essentially as specified by Stein and Moore.[22] Some as yet unresolved difficulties associated with the determination of cysteine chromatographically must delay the presentation of the whole analytical picture as determined by this method. But in several instances the determination of residual cystine and cysteine, as determined by our colorimetric method, the determination of CSA by Lavine's method, and the determination of cysteic acid give a substantially complete material balance (Table VII).

Both the experimental results and the interpretation offered here differ considerably from those of Swan,[5] but all of Swan's experiments were conducted at 100° or higher, and his principal conclusion, that a β elimination characterizes the alkaline decomposition of IX, is based largely on the observations that under comparable conditions α,α'-dimethylcystine (X) decomposes much more slowly than IX, or not at all. From Table VIII it can be seen that, while X decomposes somewhat more slowly than IX, it does decompose quite extensively at 35.2° and according to a pattern similar to that of IX; the sulfur can be accounted for satisfactorily as residual disulfide, thiol, and sulfonic acid. Since there appears to be no qualitative difference, and only a small quantitative one, between the behaviors of IX and X, there is no experimental basis for Swan's conclusion

(21) However, E. G. Howard [*J. Org. Chem.*, **27**, 2212 (1962)] and R. G. Hiskey and co-workers [*ibid.*, **29**, 3671, 3678, 3684 (1964)] have reported disulfide to thio ether transformations with alkoxide ion in anhydrous alcohol.

(22) S. Moore, D. H. Spackman, and W. H. Stein, *Anal. Chem.*, **30**, 1185 (1958).

TABLE VII

DECOMPOSITION OF CYSTINE (IX) IN AQUEOUS SODIUM HYDROXIDE AT 35.2° [a]

[NaOH], N	Time, days	[RSSR], $M \times 10^4$	[RSH], $M \times 10^4$	% decompn	Sulfur balance, %
0.105	0	13.5	0.0	...	...
	9.0	11.9	1.95	11.8	96
	14.0	11.8	2.58	12.6	97
0.526	0	14.8	0.0	...	...
	0.9	12.9	3.94	12.8	100
	2.0	12.1	5.8	18.3	102
	5.4	9.34	8.72	36.9	92.5
0.550	0	54.3	0.0	...	...
	1.0	46.9	10.8	13.6	96
	4.0 [b]	35.7	23.6	34.3	87.4

[a] No hydrogen sulfide observed at any time. [b] 6.0×10^{-4} M cysteic acid.

TABLE VIII

DECOMPOSITION OF α,α'-DIMETHYLCYSTINE (X) IN AQUEOUS SODIUM HYDROXIDE AT 35.2° [a]

[NaOH], N	Time, days	[RSSR], $M \times 10^4$	[RSH], $M \times 10^4$	% decompn	Sulfur balance, %
0.526	0	11.8	0.0	...	...
	1.0	11.3	0.9	4.3	100
	2.0	11.1	1.3	5.8	100
	5.0	10.6	2.3	10.0	100
1.08	0	365	0.0	...	...
	13.0 [b]	157	358	57.0	92.1

[a] No hydrogen sulfide observed at any time. [b] Ion-exchange column chromatography established the presence of a ninhydrin-sensitive sulfonic acid, presumably $HO_3SCH_2C(CH_3)(NH_2)$-COOH.

that IX must decompose by a β elimination since X, which is structurally incapable of undergoing a β elimination, is much more stable than IX. The results presented here support the view that both IX and X decompose by direct attack of hydroxide ion on sulfur.

Compounds IX, XIV, and X, then, are the only dithiodicarboxylic acids which appear to undergo decomposition by direct attack. In the case of XIV the separation of each carboxylate anion from its corresponding sulfur atom by three methylene groups lowers the inhibition by charge repulsion so that slow decomposition is observed. In IX and X, which invite comparison with XI, XV, and XII, the amino group somehow represses the inhibitor effect of the carboxylate anion. Clearly, neutralization of charge is not involved, for the amino group is completely uncharged in the strongly alkaline solutions, but the empirical effect is there. In the case of IX the amino group might have been expected to have some additional labilizing effect on the proton attached to the same carbon atom so that an elimination reaction would take place even more easily than in the case of XI. Empirically, however, it is seen that the damping of the inhibition of direct attack by carboxylate anion is more important than the labilization of the proton.

Finally, there is the interesting case of penicillamine disulfide (XVII) which is completely resistant to 1.09 N sodium hydroxide solution at 35.2° for at least 9 days. Compound XVII invites comparison with XII, which does decompose slowly, presumably by an elimination reaction. Since, as stated immediately above, the amino group might be expected to facilitate an elimination reaction, the fact that XVII is completely

resistant to alkali under the conditions employed indicates the likelihood of steric hindrance, a factor not previously mentioned, though it might have been in connection with XVI, which is also completely resistant to alkali. In the latter case, however, the proximity of the carboxylate anion to sulfur gives an alternative, and at least equally important (see also XV), reason for stability. Additional support for the idea that steric hindrance explains the stability of XVII is provided by the previously unrecorded facts that XVII is completely resistant to cleavage at room temperature by either zinc amalgam–acid couple or by sulfite ion. Needless to say, penicillamine itself (the thiol) develops the normal blue color with phosphotungstic acid and is oxidized to XVII.

In summary, the experimental evidence presented here suggests that many aliphatic disulfides, neutral or positively charged molecules, decompose readily in aqueous alkali by direct attack of hydroxide ion on sulfur to give thiol and sulfonic acid as the principal products. Negatively charged molecules either do not react at all, react considerably more slowly by direct attack, or, when feasible, react to give different kinds of products which can often be accounted for by an elimination mechanism. (See Table IX for a summary of results and conclusions.)

TABLE IX
SUMMARY OF RESULTS AND CONCLUSIONS[a]

No.	Compd	Product of decompn[b]	H_2S formed	Possible mode of decompn
I	$(SCH_2CH_2\overset{+}{N}Me_3)_2$	...	No	A
II	$(SCH_2COOH)_2$	Oxalic acid	Yes	B
III	$(SCHCOOH)_2$ (Me)	Pyruvic acid	Yes	B
IV	$(SCH_2CH_2OMe)_2$	O-Methyl-isethionic acid	No	A
V	$(SCH_2CH_2OH)_2$	Isethionic acid	No	A
VI	$(SCH_2CH_2NH_2)_2$	Taurine	No	A
VII	$(SCH_2CMe_2CONH_2)_2$	...	No	A
VIII	$(SCH_2CH_2COOEt)_2$	...	No	A
IX	$(SCH_2CHCOOH)_2$ (NH_2)	Cysteic acid	No	A
X	$(SCH_2C(CH_3)COOH)_2$ (NH_2)	α-Methyl-cysteic acid	No	A
XI	$(SCH_2CH_2COOH)_2$	Hydroxy-pyruvic acid (?)	Yes	B
XII	$(SCMe_2CH_2COOH)_2$	...	Yes	B
XIII	$(SCH_2CH_2SO_3H)_2$	...	...	?
XIV	$(SCH_2CH_2CH_2COOH)_2$	4-Sulfobutyric acid	No	A
XV	$(SCH_2CMe_2COOH)_2$	No decompn		
XVI	$(SCMe_2COOH)_2$	No decompn		
XVII	$(SCMe_2CHCOOH)_2$ (NH_2)	No decompn		

[a] Arranged roughly in the order of increasing stability toward NaOH. [b] Product identified other than thiol. [c] A, direct nucleophilic attack; B, elimination reaction.

Experimental Section

Materials.—We are grateful to Evans Chemetics, New York, N. Y., for generous gifts of 2-mercaptoethylamine hydrochloride, 2-mercaptoethyldimethylamine hydrochloride, and 3-mercaptopropionic acid; and to the Toni Co., Chicago, Ill., for a generous gift of 2-mercaptoethanesulfonic acid sodium salt. 2-Mercaptoethanol and 4-mercaptobutyric acid, as well as chemical intermediates not otherwise specified, were purchased as Eastman organic chemicals from Distillation Products Industries, Rochester, N. Y. L-Cystine, L-cysteine hydrochloride monohydrate, and DL-penicillamine were purchased, respectively, from Mann Research Laboratories, New York, N. Y., Calbiochem, Los Angeles, Calif., and Sigma Chemical Co., St Louis, Mo. The preparation of 3,3′-dithiodipropionic acid (XI) was described in the preceding paper.[3]

2,2′-Dithiodiethanol (V) was prepared by placing 100 g of 2-mercaptoethanol (98% pure by iodometric titration) in a flask, cooling with ice, adding 72.5 ml of 30% hydrogen peroxide dropwise with vigorous stirring (temperature below 50° internally), allowing the solution to stand overnight until thiol had completely disappeared (test with phosphotungstic acid), and removing the water under reduced pressure on a rotary evaporator to furnish 99.0 g of colorless, rather viscous liquid. A purity of 99.1% was determined by passing a sample through the Jones reductor and titrating the effluent with standard iodine. Attempts to distil the product, even at low pressure, always resulted in decomposition.

2,2′-Dithiodiethylamine (VI) dihydrochloride was similarly prepared by slow addition of 8.0 g of 30% hydrogen peroxide to a cooled, aqueous solution of 15.0 g of 2-mercaptoethylamine hydrochloride, evaporating after obtaining a negative test for thiol, suspending the crystalline mass in acetone, suction filtering, and drying over calcium chloride (yield 15.0 g).

2,2′-Methoxyethyl disulfide (IV) was prepared by an adaptation of a method of Parham, *et al.*[23] A mixture of 76 g of 2-methoxyethyl-*p*-toluenesulfonate, 5 g of potassium iodide, 60 g of sodium sulfide nonahydrate, 12 g of sulfur, and 250 ml of absolute ethanol was refluxed for 12 hr, cooled and filtered, the filter cake was washed with ethanol, the combined filtrates were evaporated, the residual liquid was diluted with water, the solution was extracted with ether, and the ether extract was dried over magnesium sulfate and evaporated to leave 23.4 g of yellow oil. Distillation at reduced pressure gave IV, bp 48–49° (0.1 mm).

A reference sample of 2-methoxyethanesulfonic acid ammonium salt was prepared by adding 25 ml of 30% hydrogen peroxide in 25 ml of glacial acetic acid to 1.0 g of IV in 6.8 ml of glacial acetic acid, heating for 1 hr at 70–80°, cooling, evaporating the acetic acid, adding concentrated aqueous ammonia, and evaporating to give 1.0 g of white, crystalline solid.

3,3′-Dithiodipropionic acid diethyl ester (VIII) was prepared by refluxing 10 g of XI and 1.5 ml of concentrated sulfuric acid in 300 ml of absolute ethanol for 16 hr in a Soxhlet apparatus with magnesium sulfate in the thimble, evaporating the ethanol under reduced pressure, taking up the residue in ether, washing with sodium bicarbonate solution followed by water, drying over magnesium sulfate, and evaporating the ether. Distillation gave 8.0 g of VIII, bp 159–160° (2.9 mm).

α,α′-Dimethylcystine (X) was prepared by following the method of Swan and Stapleton.[5]

3-Mercapto-3-methylbutanoic acid was prepared by following the method of Földi and Kollonitsch[24] and the thiol was carefully oxidized to dithiodiisovaleric acid (XII) in the usual manner. Compound XII melted at 95°.

2,2′-Dithiodiethanesulfonic acid (XIII) was prepared by adding the calculated amount of hydrogen peroxide to an aqueous solution of 2-mercaptoethanesulfonic acid sodium salt and evaporating the water until crystallization ensued. Analyses for carbon, hydrogen, and sulfur indicate that XIII disodium salt crystallizes as a dihydrate which holds water tenaciously against P_2O_5, but readily loses water of hydration on heating to 125°.

4,4′-Dithiodibutyric acid (XIV) was prepared by oxidizing the corresponding thiol carefully by hydrogen peroxide in the usual manner. Compound XIV melted at 108–108.5°.

Dithiodipivalic acid (XV) was prepared by following the method of Greene and Hagemeyer[25] who reported a melting point of 153–154°. Our product melted at 146°.

Dithiodipivalylamide (VII), mp 163–165°, was prepared from XV by the method of Greene and Hagemeyer,[25] who reported a melting point of 164–165°.

Dithiodiisobutyric acid (XVI) was prepared by an adaptation of the method of Biilmann.[26] To an aqueous solution of 66 g of

(23) W. E. Parham, H. Wynberg, and F. L. Ramp, *J. Am. Chem. Soc.*, **75**, 2065 (1953).

(24) Z. Földi and J. Kollonitsch, *J. Chem. Soc.*, 1683 (1948).

(25) J. L. Greene, Jr., and H. J. Hagemeyer, Jr., *J. Am. Chem. Soc.*, **77**, 6065 (1955).

(26) E. Biilmann, *Ann.*, **348**, 127 (1906).

potassium ethyl xanthate was added 34 g of α-bromoisobutyric acid, the solution was kept at room temperature for 24 hr, the heavy, oily layer was separated and discarded, the aqueous layer was acidified with 6 *N* HCl, the white precipitate was dissolved in 75 ml of concentrated aqueous ammonia, the solution was evaporated to a thick syrup after 48 hr, and the syrup was extracted with ether to remove xanthogenamide, somewhat diluted with water, and acidified, whereupon 1.60 g (very poor yield) of XVI separated. Biilmann[26] reported a melting point of 197°. Our product melted at 185°; by titration with standard alkali it was 95.2% pure, free of thiol. Like penicillamine disulfide (XVII), XVI cannot be reduced to the thiol by the zinc amalgam–acid couple.

Penicillamine disulfide (XVII) was prepared by dissolving 4.0 g of penicillamine in water, adding aqueous ammonia to pH 9, adding a trace of ferric chloride, aerating until the purple color faded, evaporating to dryness, and recrystallizing the white solid from aqueous ethanol (yield 3.8 g melting at 175°).

3,4-Dithiahexane-1,6-bistrimethylammonium (I) iodide was prepared by following the method of Renshaw, *et al.*[27]

Standard Procedures.—The procedure for following the alkaline decomposition of disulfides, for determination of disulfide, thiol, and hydrogen sulfide, and for the preparation of phosphotungstic acid reagent were described in the preceding paper.[3]

Isolation of 2,4-Dinitrophenylhydrazine Derivative of Hydroxypyruvic Acid (?).—3,3′-Dithiodipropionic acid (XI, 10.9466 g) was weighed into a 100-ml volumetric flask, neutralized with 4.2 g of sodium hydroxide in 10 ml of boiled and cooled distilled water, and the flask was brought to the mark with 1.5 *N* NaOH. In addition to the periodic removal of aliquots for determination of thiol, disulfide, and hydrogen sulfide, 5-ml aliquots were pipetted into test tubes containing 8 ml of *N* HCl. The solutions were filtered to free them of the voluminous, white precipitate of XI, aspirated with nitrogen to remove hydrogen sulfide, and treated with 2,4-dinitrophenylhydrazine. On cooling, an orange solid formed which was collected by suction filtration and dried *in vacuo* over P_2O_5. The pooled samples were recrystallized from ethyl acetate, mp 160–161° (23.4% N). (See Table II.)

Product Identification from the Alkaline Decomposition of 2,2′-Dithiodiethanol (V). A.—A solution of 4.47 g of V in 100 ml of 1.089 *N* NaOH was kept under nitrogen for 10 days, when analysis indicated that 53% of the disulfide had decomposed and that 2.0 g of thiol was present. Still under nitrogen, the solution was passed, rinsing with water, through a column of cation-exchange resin (Amberlite CG 120) in the hydrogen form to remove alkali, then through a column of anion-exchange resin (Amberlite CG 45) to remove acid. The final effluent was treated with 4.0 g of mercuric acetate in 100 ml of water to give a voluminous, white precipitate which, when filtered and dried, gave 3.2 g, melting at 121°. A mixture melting point with an authentic specimen of the mercuric mercaptide of 2-mercaptoethanol was not depressed.

B.—In a similar experiment 9.12 g of V in 100 ml of 1.089 *N* NaOH was kept under nitrogen for 10 days, when analysis indicated that 38% of the disulfide had decomposed. Still under nitrogen, the solution was freed of sodium hydroxide by ion exchange and the effluent was brought to 250 ml. Titration of a 5-ml aliquot with 0.0109 *N* NaOH required 20.60 ml; a total of 11.3 mequiv of acid formed from the decomposition of 22.5 mmoles of V. Concentrated, aqueous ammonia (50 ml) was added to the rest of the solution, which was then evaporated to a few milliliters of pale yellow, viscous liquid, which was examined by thin layer chromatography on silica gel H. Chromatograms which were developed with 1% acetic acid, with 4:1 butanol–acetic acid, or with 3:2 butanol–acetic acid, using bromcresol green as the indicator, gave single spots whose R_f values were identical with those given by authentic isethionic acid ammonium salt under the same conditions. When duplicate chromatograms were treated with iodine vapor, two spots were obtained in each case, whose R_f values were identical with those given by authentic 2-mercaptoethanol and V.

Product Identification from the Alkaline Decomposition of 2,2′-Dithiodiethylamine (VI).—The procedures employed were exactly the same as those described for V. In this case, however, the excess capacity of the cation-exchange resin in the hydrogen form removed 2-mercaptoethylamine and VI, as well as the sodium hydroxide. Evaporation of the effluent yielded 256 mg of white solid which, recrystallized from aqueous methanol, melted at 319–320° dec. A mixture melting point with authentic taurine was not depressed. In this case the actual decomposition of 15.1 mmoles of VI gave 2.06 mmoles of taurine recovered.

Determination of Ammonia Formed during Alkaline Decomposition of VI.—Control experiments with solutions containing 0.01 mg/milliliter of NH_4Cl, 0.41 mg/milliliter of VI dihydrochloride, or 0.10 mg/milliliter of 2-mercaptoethylamine hydrochloride showed that ammonium ion is quantitatively eluted from an 8-cm column of Amberlite CG 120, equilibrated with citrate buffer at pH 5.22, by elution with 45–55 ml of the same buffer, and that neither disulfide nor thiol (as measured by phosphotungstic acid) came through in 65 ml. The ammonia was determined by treating 5 ml of effluent with 1 ml of ninhydrin reagent,[28] heating in a boiling water bath for 15 min, cooling, diluting with 5 ml of 1:1 aqueous ethanol, and determining the optical density at 570 mμ using a Bausch and Lomb Spectronic 20. Micrograms of ammonia were read from a standard curve prepared with a dilute ammonium chloride solution.

Determination of Cysteic Acid and of α-Methylcysteic Acid.—It was considered that ion-exchange column chromatography in conjunction with ninhydrin detection[22] would provide a convenient and satisfactory way for determining the possibly complex assortment of compounds which might arise from the alkaline decompositions of cystine (IX) and of α-methylcystine (X). The details of the procedure developed will not be given here since it is not yet entirely satisfactory for cysteine. However, when aliquots of alkaline decomposition runs of IX which had been pipetted into, and brought to volume with, citrate buffer at pH 3.20 were put onto a 17-cm column of Amberlite CG 120, equilibrated with citrate buffer at pH 3.20, cysteic acid came out in the hold-up volume of the column, residual cystine was readily determined, and there was no evidence for lanthionine or 2-methylthiazolidine-2,4-dicarboxylic acid. The data in Table VII are colorimetric for IX and cysteine, and chromatographic for the single value for cysteic acid given. No attempt has been made to standardize the method for X, but when an aliquot of an alkaline decomposition run of X is treated as above something comes out in the holdup volume of the column which, as a ninhydrin-sensitive strong acid, can scarcely be anything other than α-methylcysteic acid.

Registry No.—I, 10498-85-8; IV, 10498-86-9; V, 1892-29-1; VI, 51-85-4; VIII, 1609-40-1; IX, 56-89-3; X, 4727-05-3; XI, 1119-62-6; XI DNPH derivative, 10498-90-5; XII, 10498-91-6; XIV, 2906-60-7; XVII, 312-10-7; taurine, 107-35-7.

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(27) R. R. Renshaw, P. F. Dreisbach, M. Ziff, and D. Green, *J. Am. Chem. Soc.*, **60**, 1765 (1938).

(28) Ninhydrin reagent is prepared by dissolving 1 g of ninhydrin and 0.150 g of hydrindantin in 37.5 ml of Methyl Cellosolve and adding 12.5 ml of 4 *M* acetate buffer (pH 5.5) immediately before use.