Organic Disulfides and Related Substances. IX.[1a] Symmetrical Aminothiolsulfonates as Antiradiation Drugs[1b]

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Various symmetrical aminothiolsulfonates were investigated with respect to influences of structure both on protective activity against ionizing radiation and on preparation and properties. In comparison with the thiolsulfonate obtained by oxidizing cysteamine hydrochloride, a chain of five carbon atoms instead of two or acylation of the amine by guanyl or acetyl likewise resulted in quite satisfactory preparation; protective activity ranged from none to good. Thiols or disulfides containing secondary amino groups either tended to be difficultly oxidized by hydrogen peroxide or to be over oxidized (*e.g.*, to a sulfonic or carboxylic acid); chlorinolysis afforded a promising alternative approach for a member of this class; protective action was good. Thiols containing tertiary amino groups were oxidized smoothly to thiolsulfonates which, however, were not protective. Secondary and tertiary thiols or disulfides as well as aromatic or heterocyclic disulfides containing electron-withdrawing groups, all bearing amino moieties, gave no aminothiolsulfonates with hydrogen peroxide. Stabilities of the aminosulfonates varied markedly at pH 5.4 and upon extraction as free bases.

An earlier paper described oxidation of cysteamine hydrochloride (**1**) and its N-acetyl derivative (**2**) to the symmetrical thiolsulfonates (**3** and **4**, respectively), as shown by the equation.[2] Hydrogen peroxide, far the most effective oxidant of several tried,[2] had been used by Christiansen and Dolliver for oxidation of cystamine dihydrochloride (**5**) to a presumed disulfoxide, which no doubt was actually the aminoethyl thiolsulfonate (**3**).[2,3]

Thiolsulfonates **3** and **4** seemed promising for protection against lethal effects of ionizing radiation, because thiolsulfonates ordinarily convert thiols to disulfides according to the following equation.[2]

$$RSO_2SR + R'SH \longrightarrow RSSR' + RSO_2H$$

Christiansen and Dolliver had oxidized 2-aminopropyl and 3-aminopropyl disulfide dihydrochloride, in addition to the cystamine salt (**5**), with hydrogen peroxide. Evidently compounds with primary sulfhydryl and amine functions can be oxidized nicely to thiolsulfonates with peroxide.[2,3] The present paper reports effects of chain length and of structural environment at the sulfur and nitrogen atoms on the preparations, properties, and protective activities of symmetrical thiolsulfonates. Oxidation of certain unsymmetrical disulfides has already been considered to some extent.[1a]

Variation in the N-Acyl Group.—Since oxidation of N-acetylcysteamine (**2**) had given the protective thiolsulfonate **4** smoothly, the acyl function was changed to

RSH (H_2O_2)	RSSR (H_2O_2)	RSO_2SR
R =	R =	R =
1, $Cl^-H_3N^+(CH_2)_2$	**5**, $Cl^-H_3N^+(CH_2)_2$	**3**, $Cl^-H_3N^+(CH_2)_2$
2, $AcNH(CH_2)_2$	**8**, $Cl^-H_3N^+(CH_2)_5$	**4**, $AcNH(CH_2)_2$
7, $Br^-H_2NC(:NH_2^+)NH(CH_2)_2$	**11**, n-$C_{10}H_{21}NH(CH_2)_2$	**6**, $Br^-H_2NC(:NH_2^+)NH(CH_2)_2$
10, n-$C_{10}H_{21}NH(CH_2)_2$	**14**, (2 Cl^-)3-$C_5H_4NH^+CH_2NH_2^+(CH_2)_2$	**9**, $Cl^-H_3N^+(CH_2)_5$
13, (2Cl^-)3-$C_5H_4NH^+CH_2NH_2^+(CH_2)_2$	**15**, $Cl^-(CH_3)NH_2^+(CH_2)_2$	**12**, n-$C_{10}H_{21}NH(CH_2)_2$
17, $Cl^-(CH_3)_2NH^+(CH_2)_2$		**16**, $Cl^-(CH_3)NH_2^+(CH_2)_2$
19, $Cl^-(C_2H_5)_2NH^+(CH_2)_2$		**18**, $Cl^-(CH_3)_2NH^+(CH_2)_2$
21, $CH_3O_2CCH(NH_3^+Cl^-)C(CH_3)_2$		**20**, $Cl^-(C_2H_5)_2NH^+(CH_2)_2$

The capability of certain protective agents for forming disulfide linkages involving thiol groups of tissue constituents has been suggested as an important basis for protective action.[4] Both **3** and **4** were indeed found to be "good" in protective activity (*cf.* Table I),[5] and it became important to determine for related thiolsulfonates their protective activity and chemical properties, as well as the generality of the previously used oxidative synthesis using hydrogen peroxide.[2]

guanyl. 2-Guanidinoethyl 2-guanidinoethanethiolsulfonate dihydrobromide (**6**) was of special interest because of the good protective activity of 2-guanidinoethanethiol (**7**).[6] Thiol **7**[7] was converted to the thiolsulfonate **6** (72% yield) by aqueous peroxide as previously described.[2]

Evidence for the structure of **6**, as well as for other thiolsulfonates described later, rests on preparative analogy,[2] elemental analysis, infrared spectrum, and a positive thiolsulfonate test.[8]

Of incidental interest is an effort to prepare 2-guanidinoethyl disulfide for comparison with thiolsulfonate **6**. The guanidino thiol (**7**) upon being shaken with oxygen consumed far more than required for conversion to the disulfide and 2-guanidinoethanesulfonic acid resulted in

(1) (a) Paper VIII: L. Field, H. Härle, T. C. Owen, and A. Ferretti, *J. Org. Chem.*, in press; (b) reported in part at the 141st National Meeting of the American Chemical Society in Washington D. C., March 26–29, 1962 (Abstracts, p. 31N), and at the Southeastern Regional Meeting of the American Chemical Society, Gatlinburg, Tenn., Nov. 1–3, 1962; (c) Texaco Fellow in Chemistry, 1961–1962; results in part are abstracted from the Ph.D. dissertation of R. R. C., Vanderbilt University, 1963; (d) research done partly during leave from the College of Technology, Liverpool, England, and partly at that College under NATO support.

(2) L. Field, T. C. Owen, R. R. Crenshaw, and A. W. Bryan, *J. Am. Chem. Soc.*, **83**, 4414 (1961).

(3) W. G. Christiansen and M. A. Dolliver, U. S. Patent 2,242,236 (1941); *Chem. Abstr.*, **35**, 5647 (1941).

(4) *Cf.* A. Pihl and L. Eldjarn, *Pharmacol. Rev.*, **10**, 437 (1958).

(5) Private communication from Drs. T. R. Sweeney and D. P. Jacobus of the Walter Reed Army Institute of Research, Washington, D. C.

(6) Formed by isomerization of the protective agent S-(2-aminoethyl)-isothiuronium bromide hydrobromide at blood pH: R. Shapira, D. G. Doherty, and W. T. Burnett, Jr., *Radiation Res.*, **7**, 22 (1957).

(7) J. X. Khym, R. Shapira, and D. G. Doherty, *J. Am. Chem. Soc.*, **79**, 5663 (1957).

(8) An acidic reaction caused by formation of a sulfinic acid upon treatment with a thiol such as **1** or *p*-toluenethiol: $RSO_2SR + R'SH \longrightarrow RSO_2H + RSSR'$; *cf.* D. Barnard and E. R. Cole, *Anal. Chim. Acta*, **20**, 540 (1959)

78% yield. Since oxygen usually oxidizes thiols only to disulfides, the alkaline medium associated with the guanidino group must have effected S–S cleavage to other readily oxidizable species.[9]

Variation in Chain Length.—Since 5-aminopentyl disulfide dihydrochloride (8) was known,[10] it afforded a means for studying the activity, synthesis, and properties of a thiolsulfonate with a long chain. Oxidation of disulfide 8 to thiolsulfonate **9** proceeded fairly well (49% yield) with aqueous peroxide.

Variation in Type of Amine.—To exemplify a secondary amine, 2-(*n*-decylamino)ethanethiol (**10**) hydrochloride was studied in the hope that the long chain would enhance diffusion of the thiolsulfonate (**12**) salt across membrane barriers. Initial oxidations with aqueous peroxide[2] gave no indication of thiolsulfonate formation. The product had strong absorption at 2450 cm.$^{-1}$, which led to the belief that the thiol had survived. Ultimately, however, this absorption was shown to be characteristic of the hydrochloride of disulfide **11**. Oxidation of thiol **10** to disulfide **11** or its salts actually proceeded readily. Attempts to continue oxidation to thiolsulfonate **12** gave back disulfide, however, often accompanied by 2-(*n*-decylamino)ethanesulfonic acid. These failures did not result merely from insolubility of salts of disulfide **11**, although insolubility is characteristic, because salts and solvents were found which gave complete or at least partial solubility. This instance is therefore one in which the previous oxidation[2] with peroxide fails.

Douglass and Farah have converted disulfides to thiolsulfonates using chlorine.[11] This method failed for conversion of N-acetylcystamine to thiolsulfonate **4**,[2] but was tested for conversion of cystamine salt **5** to its thiolsulfonate **3** as a model synthesis for the *n*-decylaminoethyl thiolsulfonate (**12**); **3** resulted in 23% yield (a recheck with N-acetylcystamine failed). Even better results occurred with the *n*-decylaminoethyl disulfide (**11**) dihydrochloride, and the salt of thiolsulfonate **12** was obtained in 85% yield.

Oxidation with aqueous peroxide[2] of another secondary amine, 2-[3-pyridylmethylamino]ethanethiol dihydrochloride (**13**),[12] gave nicotinic acid hydrochloride as the only isolable product (30%), and use of the disulfide tetrahydrochloride (**14**) resulted only in recovered **14**; the procedure of Douglass and Farah[11] gave an unidentified material.

Aqueous peroxide[2] was used successfully with a third secondary amino disulfide, 2-(methylamino)ethyl disulfide dihydrochloride (**15**). Thiolsulfonate **16** resulted in 56% yield. Again the secondary amino compound was anomalous. A much longer reaction time was necessary, and appreciable over oxidation to a sulfonic acid occurred. Unless excess peroxide was used, a difficultly separable mixture of disulfide **15** and thiolsulfonate **16** resulted. 2-(Methylamino)-ethanesulfonic acid was separated from the desired thiolsulfonate (**16**) by conversion of hydrochloride **16** into the *p*-toluenesulfonate salt which, unlike the sulfonic acid, was soluble in 2-propanol.

One may conclude that oxidation with aqueous peroxide is far less predictable with secondary than with the primary aminodisulfides. A delicate balance seems to exist between the ease of oxidation to thiolsulfonate and of further oxidation of the thiolsulfonate, a balance which may be upset by slight changes in structure.

In view of the difficulties with the three secondary amines, results with tertiary amines in the usual peroxide oxidation[2] came as a pleasant surprise. 2-(Dimethylamino)ethanethiol hydrochloride (**17**) gave the thiolsulfonate (**18**) smoothly in 78% yield, and the diethyl analog (**19**) gave the thiolsulfonate (**20**) in a yield of about 59%.

Variation in the Type of Thiol Function.—As stated, primary thiols **1** and **2** were oxidized smoothly by aqueous peroxide to thiolsulfonates.[2] Extension of the usual oxidation[2] to the secondary thiol 1-amino-2-propanethiol gave no isolable thiolsulfonate. The product was first thought to be a thiolsulfinate, as shown in the equation, but attempts to demonstrate a thiolsulfinate structure were inconclusive. Since

$$H_2NCH_2CH(CH_3)SH \xrightarrow{H_2O_2,\ HCl} Cl^-H_3N^+CH_2CH(CH_3)S(O)SCH(CH_3)CH_2NH_3^+Cl^-$$

the yield was low and efforts to improve it were unavailing, further work seemed unjustified. The infrared spectrum indicated the product may have been a mixture of a sulfonic acid and disulfide.

Two unsymmetrical *tert*-alkylaminoethyl disulfide salts were oxidized earlier to thiolsulfonates, but when an alkoxycarbonyl moiety was present on the same carbon atom as the amine function no thiolsulfonate could be isolated.[13] Nevertheless, efforts to form symmetrical thiolsulfonates in which the sulfur function was attached to a tertiary carbon atom were conducted with the D,L-penicillamine ester **21**, because it seemed the most conveniently available tertiary thiol likely to afford a worthwhile antiradiation drug. Results were quite unpromising. The earlier results mentioned suggest that presence of the β-alkoxycarbonyl function may be more significant as a cause of the difficulty than attachment of sulfur to the tertiary carbon atom.

In the usual procedure with peroxide,[2] its molar proportion to the ester **21** was varied (1.5–1.75), as well as the time of reaction (6–24 hr.). Hygroscopic glasses resulted. The pH dropped from about 4 to 1 or less, indicating formation of sulfonic or sulfinic acids. Titration with alkali also showed formation of much acid. Neither chromatography nor efforts to precipitate a sparingly soluble salt (with seven strong organic acids) availed in efforts to isolate a solid thiolsulfonate. Oxidation[2] of ester **21** as its *p*-toluenesulfonate instead of its hydrochloride, and oxidation of penicillamine disulfide or its ester as hydrochlorides gave similar results. Similar outcomes in oxidation of ethyl esters of cysteine or cystine hydrochlorides confirm the supposition that the β-alkoxycarbonyl function does indeed contribute to the poor results. Additionally, however, the mechanism of action of the aqueous peroxide probably plays a role, because cystine can be oxidized smoothly with performic acid to the thiolsulfonate[13] but use of aqueous peroxide[2] resulted only in recovery of cystine.

As an example of an aminoaryl disulfide, o-aminophenyl disulfide dihydrochloride was treated with

(9) *Cf.* A. Schöberl and A. Wagner, "Methoden der Organischen Chemie (Houben-Weyl)," Vol. 9, E. Muller, Ed., 4th Ed., G. Thieme Verlag, Stuttgart, p. 75.

(10) G. Losse and K-H. Richter, *J. prakt. Chem.*, **13**, 23 (1961).

(11) I. B. Douglass and B. S. Farah, *J. Org. Chem.*, **24**, 973 (1959).

(12) T. P. Johnston and A. Gallagher, *ibid.*, **27**, 2452 (1962).

(13) R. Emilozzi and L. Picha, *Bull. soc. chim. France*, 1887 (1959).

aqueous peroxide;[2] the system was homogeneous. Evidently the ammonium salt was too highly deactivating, because starting material resulted (78%); under forcing conditions (65°) only 15% of disulfide salt was recovered and other products were intractable tars. The supposition of deactivation by electron withdrawal is supported by a report of the oxidation of *o*-acetamidophenyl disulfide to the thiolsulfonate ("disulfoxide") with hydrogen peroxide.[14]

A heterocyclic disulfide also failed to give a thiolsulfonate, when aqueous hydrogen peroxide was used in the usual way,[2] probably again because of deactivation by electron withdrawal. Thus 2-pyridyl disulfide dihydrochloride gave only pyridine-2-sulfonic acid (25%) and starting material; disulfide salt also was recovered after attempted chlorinolysis.[11]

Relative Stability of Thiolsulfonates.—An earlier report suggested that the aminoethyl base (**3** free base) was unstable, although presence of excess alkali made this conclusion uncertain.[15] The relative stabilities of this and related free bases were of considerable interest, because of possible correlation with radioprotective activity, as well as because of obvious relevance to synthesis and handling.

Initial experiments bore out the instability of **3** free base in solution: (a) Neutralization of **3**, extraction of the base, and reconversion to **3** resulted in recovery only of 9%. Since marked solubility in water of the base might explain the low recovery, an effort also was made to convert the dihydrochloride **3** essentially to free base with silver acetate and then to reconvert the base to **3**; no **3** was recovered, but the experiment proved irrelevant because silver acetate itself destroyed the acetyl analog **4**. (b) Titration of dissolved **3** with alkali resulted in decreases of pH shortly after each addition, until $^4/_3$ equiv. of alkali per mole of **3** had been added (a similar fading of pH occurred in titration of the guanidino thiolsulfonate **6**). Moreover, neutralization of **3** with 2 equiv. of alkali gave the same pH of 9 whether or not the solution was boiled after neutralization. Alkaline decomposition of **3** free base might occur according to the equation[16]

$$3RSO_2SR + 4KOH \rightarrow 4RSO_2K + (RS)_2 + 2H_2O$$

(c) Use of a technique[17] in which alkali is added at constant rate and an inflection in the curve of pH taken as indicating decomposition of a thiolsulfonate resulted with **3** in an inflection at about pH 5.5. This result suggested abnormal consumption of alkali and thus decomposition of **3**, at about pH 5.5. The technique was not applied to other thiolsulfonates because there seemed reason to believe that comparison of stabilities would be complicated by such factors as strength of the alkali and rate of its addition.

The instability of **3** at pH 5.5 was not characteristic of all thiolsulfonates. The acetyl derivative **4** survived quantitatively after 48 hr. in water, although at pH 9 overnight it gave N,N′-diacetylcystamine in high yield and no **4** was recovered.

At this point, the best technique for comparison of stabilities seemed to lie in determination of the rate of alkali consumption at constant pH. Survival of thiolsulfonates at blood pH was of special interest, but some seemed likely to decompose too rapidly for good comparison. Studies therefore were done at pH 5.4. Results are shown in Fig. 1.

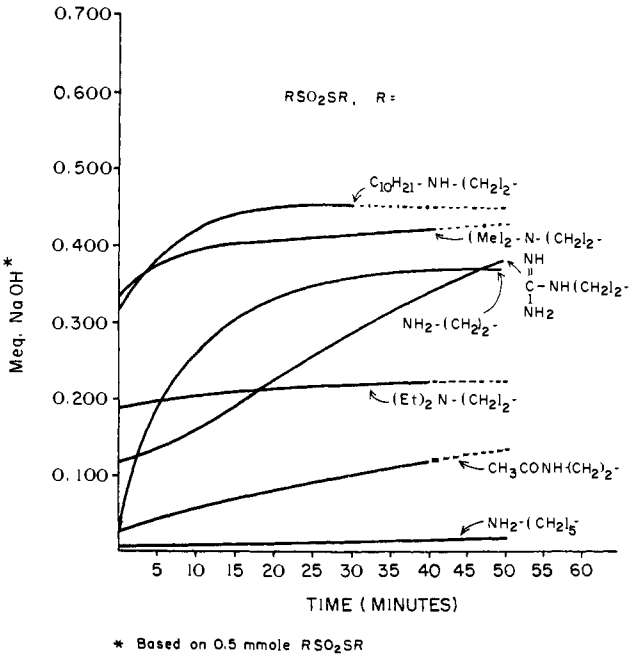

Fig. 1.—Alkali added to maintain pH 5.4, as a function of time, for aminothiolsulfonates in solution.

The curves of Fig. 1 were hard to interpret because of the unknown role played by such factors as the relation of alkali consumed to amine basicity, and the probable disproportionation of sulfinic acids produced by hydrolysis, *e.g.*[18]

$$3RSO_2SR + 2H_2O \rightarrow 4RSO_2H + RSSR$$
$$3RSO_2H \rightarrow RSO_2SR + RSO_3H + H_2O$$

However, a curve of slight slope in Fig. 1 ought to show greater stability than one of considerable slope (total consumption of alkali probably should be disregarded because of entanglement with different basicities of amines). The relative stabilities suggested in Table I thus seem reasonable. The stabilities were substantiated in the main by neutralizing the salts, extracting the bases, and reconverting the bases to the salts; results are included in Table I. Complications enter the interpretation of the extraction experiments also, even though the practical stabilities are represented; for instance, distribution coefficients of the bases undoubtedly influence the apparent stabilities, especially with the guanidino thiolsulfonate **6**.

Instability of the thiolsulfonate free bases could have been a consequence of intra- or intermolecular reactions of the amino and thiolsulfonate moieties. To test this possibility, amines were allowed to react with thiolsulfonates to determine whether reactions of the kind shown in the equation were feasible in model systems.

$$RSO_2NHR' + RSH \leftarrow RSO_2SR + R'NH_2 \rightarrow RSO_2H + RSNHR'$$

The fact that no pure products could be isolated suggests that hydrolytic and not amination reactions are the principal causes of decomposition.

(14) R. Child and S. Smiles, *J. Chem. Soc.*, 2696 (1926).
(15) D. Cavallini, C. De Marco, and B. Mondovi, *Giorn. Biochem.*, **2**, 338 (1953); *Chem. Abstr.*, **49**, 13897 (1955).
(16) See ref. 9, p. 690 (A. Schöberl and A. Wagner).
(17) G. E. Utzinger, *Experientia*, **17**, 374 (1961).
(18) L. Bauer and J. Cymerman, *J. Chem. Soc.*, 109 (1950).

Table I

Protective Activities[a] and Suggested Relative Stabilities of Thiolsulfonates

Relative stability inferred from Fig. 1	Recovery of hydrochloride after extraction, %[d]	Protection against radiation[b]	Drug dose, mg./kg.	Vehicle of administration[c]
Most stable				
$[Cl^-(C_2H_5)_2{}^+NH(CH_2)_2]_2SO_2S$ (**20**)	67 (66)[e]	None (0)	51–150	PO$_4$ buffer, pH 6.2
$[Cl^-(CH_3)_2N^+H(CH_2)_2]_2SO_2S$ (**18**)	51 (43)[e]	None (0)	351–750	Soln. in H_2O
$[Cl^-H_3N^+(CH_2)_5]_2SO_2S$ (**9**)	38	None (0)	50 or less	Saline soln.
Intermediate stability				
$[AcNH(CH_2)_2]_2SO_2S$ (**4**)	100[e]	Good (++++)	351–750	Soln. in H_2O
$[Cl^-n\text{-}C_{10}H_{21}NH_2(CH_2)_2]_2SO_2S$ (**12**·2 HCl)[f]	[g]	Good (++++)	50 or less	Susp. CMC/Tw
Least stable				
$[Br^-H_2NC(:N^+H_2)NH(CH_2)_2]_2SO_2S$ (**6**)	0	Fair (+++)	51–150	Soln. in H_2O
$[Cl^-H_3N^+(CH_2)_2]_2SO_2S$ (**3**)	9	Good (++++)	351–750	Soln. in H_2O

[a] The methylaminoethyl ester **16** was rated "Good (++++)" when administered intraperitoneally in aqueous solution (adjusted to pH 5.5) at the level of 150–350 mg./kg. [b] See ref. 5. On the activity scale used, cysteamine is rated "Good (++++)" and N-acetylcysteamine is rated "Fair (+++)". [c] The saline solution was physiological; CMC/Tw means that the compound was suspended in a physiological saline solution containing 0.2% methylcellulose (4000 centipoises) and 0.4% Tween 180. [d] Extraction performed 5 min. after neutralization instead of immediately, for further comparison of stabilities. [e] Recovered as **4** after 48 hr. in water (initial pH *ca.* 6). [f] Comparison of stability vitiated by the necessity of using 62% aqueous ethanol to effect solution. [g] Not done because very sparing solubility of the **12**·2HCl in water prevented an experiment similar to the others.

Protective Activities.—Compounds were administered intraperitoneally to mice using the vehicle shown in Table I. The mice were tested for 30-day survival against lethal radiation of 1000 r. (100 r./min.) from a cobalt-60 source or 800 r. from an X-ray source (GE 300-kv. Maxitron; 35 r./min., filtered through copper (2 mm.) and aluminum (0.25 mm.)). These irradiations are of comparable relative biological effectiveness. Protective activities are given in Table I on a scale of None (0), Slight (+ or ++), Fair (+++), or Good (++++) and are correlated with similar ratings for two well known standards. Unless otherwise specified, the pH was adjusted to 7.4; stabilities during about 15 min. required for preparation and injection of the solutions evidently sufficed for a high degree of protection, but further studies at lower pH probably would be worthwhile.

Experimental[19]

2-Guanidinoethyl 2-Guanidinoethanethiolsulfonate Dihydrobromide (6).—S-(2-Aminoethyl)isothiuronium bromide hydrobromide (AET)[20] (11.24 g., 40.0 mmoles) was added rapidly to a solution of barium hydroxide (octahydrate, 12.6 g., 40.0 mmoles) in water (200 ml.). After 10 min., sulfuric acid (4 g.) in ice–water (20 ml.) was added. A trace of iodine and hydrogen peroxide (6.7 ml., 9.75 *M*, 65.3 mmoles) were added to an aliquot of 0.9 of the total mixture. After 2 days at 30°, precipitate ($BaSO_4$) was removed and the solution was evaporated below 40°. Dissolution of the resulting sirup in acetic acid and storage at 0° gave the crystalline salt **6** (4.0 g., 52%; m.p. 162–164°); recrystallization from aqueous acetic acid gave **6** with m.p. 164–165° (Kofler); positive thiolsulfonate test,[8] strong infrared absorption at 1120, 1308, and 1320 cm.$^{-1}$. Further less pure **6** (1.5 g., 20%; m.p. 159–163°, Kofler) was obtained by evaporating the mother liquors and recrystallizing from acetic acid–ethanol.

Anal. Calcd. for $C_6H_{18}Br_2N_6O_2S_2$: C, 16.75; H, 4.22; Br, 37.15; N, 19.54; S, 14.91. Found: C, 16.84; H, 4.31; Br, 37.34; N, 19.61; S, 15.21.

In the formation of 2-guanidinoethanesulfonic acid, solutions of AET (1.4 g.) and barium hydroxide (octahydrate, 1.57 g.) were placed in two arms of an apparatus which was flushed with oxygen and sealed. When the solutions were mixed and agitated, no absorption of oxygen took place for 45 min.; however, in the ensuing 2 hr. 140 ml. (30°, 760 mm.) was absorbed. Acidification with sulfuric acid, filtration, and evaporation of the filtrate below 40° gave an oil. Addition of ethanol gave a clear solution which deposited overnight 0.65 g. (78%) of the sulfonic acid, m.p. 266–268° (Kofler); recrystallization from aqueous ethanol gave m.p. 267–268° (Kofler).

Anal. Calcd. for $C_3H_9N_3O_3S$: C, 21.55; H, 5.42. Found: C, 21.42; H, 5.19.

5-Aminopentyl 5-Aminopentanethiolsulfonate Dihydrochloride (9).—By means of the procedure used in preparing **3**,[2] 5-aminopentyl disulfide dihydrochloride (**8**, 4.18 g., 13.5 mmoles)[10] was oxidized with hydrogen peroxide (2.7 ml., 10.0 *M*, 27 mmoles). Repeated recrystallization from absolute alcohol gave 2.25 g. (49%) of slightly hygroscopic **9** with a constant m.p. of 197–198° (sealed capillary); the infrared spectrum had strong absorption at 1325 and 1130 cm.$^{-1}$, and a positive thiolsulfonate test was obtained.[8]

Anal. Calcd. for $C_{10}H_{26}Cl_2N_2O_2S_2$: C, 35.18; H, 7.68; Cl, 20.77; N, 8.21; S, 18.78. Found: C, 35.23; H, 7.73; Cl, 20.55; N, 8.07; S, 18.87.

Attempted Preparation of 2-(*n*-Decylamino)ethyl 2-(*n*-Decylamino)ethanethiolsulfonate (12) Salts with Hydrogen Peroxide.—To clarify the apparent resistance of 2-(*n*-decylamino)ethanethiol (**10**)[2a] to oxidation (*cf.* Discussion), hydrogen peroxide (1.1 ml., 9.75 *M*, 10.7 mmoles) in water (8 ml.) was added to a solution of **10** (4.34 g., 20 mmoles) and a trace of iodine in acetic acid (13 ml.) at 0–5°. After 1 hr. at 0–5° and 20 hr. at 30°, evaporation below 40° gave oil (5.6 g.) which crystallized to presumed disulfide (**11**) diacetate. Two portions (0.6 g. each) dissolved in aqueous acetic acid gave with a slight excess of hydrochloric or hydriodic acid precipitates which were recrystallized from aqueous acetic acid as the disulfide (**11**) dihydrochloride (0.4 g., 73%), m.p. 262–264° (Kofler), and dihydriodide (0.4 g., 53%), m.p. 275° (dec., Kofler). The same dihydriodide (0.635 g., 77%) was obtained after iodometric titration of thiol **10**; (oxidation equiv. of **10**: calcd. 217, found 220).

The infrared spectrum of the disulfide (**11**) dihydrochloride showed a strong band at 2450 cm.$^{-1}$ not shown by other salts, or by **11**; this hydrochloride was identical with those obtained by oxidation of **10**·HCl in the usual way.[2] All spectra were otherwise consistent with the proposed structures.

Attempts failed to oxidize salts of **11** to salts of **12** in the usual way.[2] More vigorous conditions simply gave 2-(*n*-decylamino)-ethanesulfonic acid, *e.g.*, disulfide (**11**) dihydrochloride (1.7 g.) and hydrogen peroxide (2 ml., 9.75 *M*) in acetonitrile (20 ml.)–water (20 ml.) kept at 70° for 20 hr. gave no crystals upon cooling (showing complete consumption of the sparingly soluble **11** salt). Evaporation of acetonitrile and recrystallization of the precipi-

(19) Melting points were taken in a capillary tube and are corrected (ASTM-specification thermometer), unless otherwise specified. Analyses were by Galbraith Microanalytical Laboratories, Knoxville, Tenn., except for **16** which was by the courtesy of the Organic Chemistry Department of the University of Liverpool. Evaporation of solvents for isolation of products was effected under reduced pressure, usually by means of a rotary evaporator.

(20) Kindly furnished by Dr. T. R. Sweeney of the Walter Reed Army Institute of Research, Washington, D.C.

tate gave the sulfonic acid (1.0 g., 56%), m.p. 220–225° (Kofler), unchanged by further recrystallization (water, aqueous methanol, 2-propanol).

Anal. Calcd. for $C_{12}H_{27}NO_3S$: C, 54.30; H, 10.25. Found: C, 54.16; H, 10.19.

Homogeneous systems (or largely so) resulted upon attempts to oxidize thiol **10** with aqueous hydrogen peroxide using various media: acetic acid, **10** *p*-toluenesulfonate in ethanol–water or acetonitrile–water, or disulfide **10** hydrochloride in acetonitrile–water at 70°. Disulfide **11** or its salts always resulted (*ca.* 40–94%), together with variable amounts of the sulfonic acid.

2-Aminoethyl 2-Aminoethanethiolsulfonate Dihydrochloride (3) by Chlorine Oxidation.—In a procedure like that of Douglass and Farah,[11] liquid chlorine (1.89 g., 26.6 mmole) was allowed to vaporize into a suspension at 0° of cystamine dihydrochloride **5** (3.0 g., 13.3 mmoles) in 8 ml. of methylene chloride and 0.76 ml. (13.3 mmoles) of glacial acetic acid. The resulting suspension was stirred at −5° for 10 min. and then was treated with 0.48 ml. (26.6 mmole) of water. The mixture then was kept at *ca.* 20 mm. for 2.5 hr. and 10 ml. of acetic acid was added. After 24 hr. at *ca.* 25°, filtration gave 2.5 g. (73%) of solid **3** containing much **5**. One recrystallization from aqueous acetic acid gave 0.8 g. (23%) of pure **3**, m.p. 162° dec.; the infrared spectrum was identical with that of authentic **3**.[2]

N,N′-Diacetylcystamine (3.6 g.) was treated exactly like **5**. After evacuation (20 mm.), addition of 1-butanol and chilling gave hygroscopic crystals (1.9 g.), m.p. 126–129°; the product was not **4**, nor could **4** be isolated.

2-(*n*-Decylamino)ethyl 2-(*n*-Decylamino)ethanethiolsulfonate (12) Dihydrochloride by Chlorine Oxidation.—A stirred suspension of 19.95 g. (39.4 mmoles) of 2-(*n*-decylamino)ethyl disulfide (**11**) dihydrochloride in methylene chloride (40 ml.) containing glacial acetic acid (2.26 ml., 39.4 mmoles) was cooled to 3°. Chlorine (5.6 g., 3.6 ml., 78.8 mmoles) then was introduced as a gas below the surface at such a rate as to keep the temperature at −2° to 5°. The mixture then was stirred for 10 more min. at 0°, after which water (1.42 ml. 78.8 mmoles) was added slowly. The mixture was stirred at *ca.* 25° for 2 hr. Ethanol (300 ml.) was added and the mixture was chilled and rubbed. Filtration gave 17.9 g. (85%) of solid with m.p. 198–200° dec. Recrystallization from alcohol–water gave **12** dihydrochloride having a constant m.p. of 204–205° dec.; positive thiolsulfonate test,[8] strong infrared bands at 2450, 1330, 1148, 1138, 1125 cm.$^{-1}$.

Anal. Calcd. for $C_{24}H_{54}Cl_2N_2O_2S_2$: C, 53.60; H, 10.12; Cl, 13.19; N, 5.21; S, 11.93. Found: C, 53.52; H, 10.03; Cl, 13.05; N, 5.23; S, 12.03.

Oxidation of 2-[3-Pyridylmethylamino]ethanethiol Dihydrochloride.—The free base of thiol **13**[12] was dissolved in water containing 2 molar proportions of hydrochloric acid. After treatment of the homogeneous solution with hydrogen peroxide in the usual way,[2] nicotinic acid hydrochloride was the only isolable product (30%); its identity was established by its infrared spectrum and by mixture m.p. with nicotinic acid. The mother liquor gave a strong positive test for sulfate ion (barium chloride) indicating cleavage of the carbon–sulfur bond. Repetition with the disulfide tetrahydrochloride (**14**) gave only recovered disulfide salt (63%).

When 5.05 g. of the disulfide salt (**14**) was oxidized with chlorine,[11] a product of unknown structure (1.0 g.) resulted; m.p. after recrystallization from methanol, 242–244° dec.; the thiolsulfonate test was negative.[8]

Anal. Found: C, 37.00; H, 5.34; Cl, 16.91.

2-(Methylamino)ethyl 2-(Methylamino)ethanethiolsulfonate Dihydrochloride (16).—Hydrogen peroxide (19 ml. of 9 *M*, 171 mmoles, diluted with 20 ml. of water) was added dropwise during 4 hr. to a stirred ice-cold solution of 2-(methylamino)ethyl disulfide dihydrochloride (**15**; m.p. 210–211°; 20.0 g., 79.0 mmoles) in water (70 ml.). The mixture was allowed to warm to *ca.* 30° overnight and then to stand for 5 days.[21] Water was removed by evaporation (40°). Two 60-ml. portions of acetic acid were added and similarly evaporated. A methanol solution of the residue deposited crystals during 10 hr. Recrystallization from methanol gave the pure thiolsulfonate (**16**; 3.2 g., 15%). m.p. 149–150°; positive thiolsulfonate test[8]; strong infrared absorption at 1315, 1300, 1260, and 1130 cm.$^{-1}$.

Anal. Calcd. for $C_6H_{18}Cl_2N_2O_2S_2$: C, 25.26; H, 6.36; N, 9.82; S, 22.48. Found: C, 25.42; H, 6.35; N, 9.73; S, 22.21.

Further crystallization of mother liquors, induced by evaporation or by dilution with 2-propanol, gave mixtures (A) which could not be separated by crystallization. Such a mixture (20 g.) was oxidized with more hydrogen peroxide (5 ml.) as before. *p*-Toluenesulfonic acid monohydrate (27 g.) was added and hydrochloric acid was removed by evaporation; three 60-ml. portions of water and one of 2-propanol then were added and evaporated. Addition of 500 ml. of 2-propanol precipitated 2-(methylamino)ethanesulfonic acid, which was removed. Saturation of the filtrate with dry hydrogen chloride gas precipitated almost pure **16**, which was recrystallized from methanol, 9.3 g. (41%), m.p. 148–149°.

Similar treatment of 5 g. of the mixture (A) with *p*-toluenesulfonic acid monohydrate (7 g.) gave 2-(methylamino)ethanesulfonic acid (1.1 g.) and a mixture of hydrochlorides of **15** and **16** which co-crystallized from alcohol and acetone, but was cleanly separated by paper chromatography (Whatman No. 4 paper; solvent EtOH–H_2O–H_2SO_4, vols. 75: 25: 0.1; spots developed by ninhydrin or 2,4-dinitrofluorobenzene) into two components identical in R_f value with **15** and **16**. No third spot appeared which could be attributed to a thiolsulfinate.

2-(Dimethylamino)ethyl 2-Dimethylaminoethanethiolsulfonate Dihydrochloride (18).—About 0.1 of a solution of 92 ml. of 30% hydrogen peroxide (0.954 mole) in 99 ml. of water was added rapidly with stirring to a chilled solution (2°) of 90 g. (0.636 mole) of 2-dimethylaminoethanethiol hydrochloride (**17**) in 133 ml. of water containing a trace of potassium iodide. The remaining peroxide then was added dropwise so that the temperature remained below 20°. After 24 hr. at *ca.* 30°, evaporation below 43° gave a semisolid. Crystallization from methanol–acetone gave 77.8 g. (78%) of solid, m.p. 185–187° dec. Recrystallization gave **18** with constant m.p. 189° dec.; positive thiolsulfonate test,[8] strong infrared absorption at 1340, 1175, 1150, and 1138 cm.$^{-1}$.

Anal. Calcd. for $C_8H_{22}Cl_2N_2O_2S_2$: C, 30.67; H, 7.08. Found: C, 30.59; H, 7.17.

2-(Diethylamino)ethyl 2-(Diethylamino)ethanethiolsulfonate Dihydrochloride (20).—2-(Diethylamino)ethanethiol hydrochloride (**19**; 75 g.) was oxidized as described for the preparation of **18**. The crude thiolsulfonate (**20**), an oil, crystallized from methanol (100 ml.) to give 48.3 g. (59%) of solid, m.p. 100–135°. Several recrystallizations from mixtures of methanol with acetone or carbon tetrachloride gave **20** with constant m.p. 166–168° dec. However, salt **20** becomes solvated by methanol to give a solid which when dried under reduced pressure had m.p. 85–91°; an aqueous solution dried under reduced pressure gave a glass. Pure unsolvated **20** was obtained by drying the solid obtained by recrystallization from methanol–acetone under vacuum over phosphorus pentoxide at 100° for 1.5 hr.; m.p. 170° dec.; positive thiolsulfonate test,[8] strong infrared absorption at 1338 and 1140 cm.$^{-1}$.

Anal. Calcd. for $C_{12}H_{30}Cl_2N_2O_2S_2$: C, 39.01; H, 8.19; Cl, 19.20; N, 7.58; S, 17.36. Found: C, 39.04; H, 8.32; Cl, 19.30; N, 7.42; S, 17.35.

Oxidation of 1-Amino-2-propanethiol Hydrochloride.—Oxidation of this thiol (2.56 g., 20.0 mmoles)[20] by hydrogen peroxide (30.0 mmoles) as usual[2] gave a crystalline but very hygroscopic product (0.360 g.) with m.p. 210–213° dec. (sealed capillary).

Anal. Calcd. for the thiolsulfinate $Cl^-H_3N^+CH_2CH(CH_3)$-$S(O)SCH(CH_3)CH_2NH_3^+Cl^-$, $C_6H_{18}Cl_2N_2OS_2$: C, 26.77; H, 6.74; S, 23.81. Found: C, 27.25; H, 6.86; S, 24.27.

If the product *were* the thiolsulfinate, the yield of 0.36 g. would have been 13%. Efforts to increase the yield using equimolar amounts of peroxide and the disulfide salt resulted only in recovery of the disulfide salt (49%); the infrared spectrum indicated that residual material probably was the sulfonic acid; a similar result ensued when the experiment was repeated on a steam bath.

Chlorine oxidation[11] resulted only in recovery of unchanged disulfide dihydrochloride (20%) and strongly acidic wax.

Relative Stability of Thiolsulfonates. (a) Reaction Time at pH 5.4.—Each thiolsulfonate salt or amide (**4**) (0.5 mmole) was dissolved in water (35 ml.), except that **12** dihydrochloride was used in 62% ethanol–38% water because of sparing solubility in water. By means of a pH-stat (Automatic Titrator Type TTT1C, Radiometer, Copenhagen), 0.138 *N* sodium hydroxide was added as rapidly as possible until the pH became 5.4 and there remained at least 5 sec. Depending on the amount of

(21) The usual time for completion of reaction (*ca.* 24 hr.) was insufficient; evaporation (40°) at this point resulted in an explosion, presumably owing to unconsumed peroxide.

alkali required, this time was 15 sec. to 6.5 min. (11 min. for 12 dihydrochloride). The time at which pH 5.4 was reached was taken as zero time for Fig. 1. More alkali then was added automatically to maintain pH 5.4, and consumption (including that added before zero time) was plotted *vs.* time as shown in Fig. 1.

(b) Isolation and Re-acidification of Free Bases.—Each thiolsulfonate (*ca.* 1 mmoles) was added to water (*ca.* 10 ml.)–chloroform (20 ml.). The water contained exactly enough sodium hydroxide to convert all ammonium groups to their free bases. The chloroform extract was separated and the aqueous layer was extracted with two more 20-ml. portions of chloroform. Each of the three chloroform extracts was immediately extracted with the same portion of hydrochloric acid (an amount of *N* acid in 5 ml. of water equiv. to the acid originally neutralized). The acid extract was evaporated to dryness. "Recovery, %" (Table I) is based on the amount obtained after drying to constant weight under reduced pressure. Infrared spectra of all hydrochlorides isolated corresponded excellently with those of starting material, showing that products were pure.

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The Stability of Tepa and Other Aziridine Chemosterilants

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Aziridine compounds, known for some time to be cancer-inhibiting agents, are useful as insect chemosterilants. Effective utilization of these chemicals required knowledge of their stability under different conditions. Investigations employing four different analytical procedures disclosed a high vulnerability of the aziridine chemosterilants to even mildly acidic aqueous solutions. Tepa in acidic or neutral solutions gave a stable degradation product, which with the aid of n.m.r. was identified as ethylenimine. The stability of ethylenimine in buffered solutions was found to decrease with increasing pH, whereas the stability of tepa increased with increasing pH of the medium. Regulation of the pH of insect diets resulted in a marked increase in activity of added chemosterilants.

The biological and physiological properties of aziridine derivatives active as cancer chemotherapeutic agents have been investigated thoroughly during the past ten years,[1] but only scattered and often conflicting reports of their chemical stability and reactivity have appeared. Interest in these compounds was intensified recently by the discovery of their activity as insect chemosterilants (antifertility agents) and their potential usefulness as new and powerful tools for insect control and eradication.[2]

Chemicals screened for sterilant activity were administered by several routes, including incorporation in the diet and addition to the drinking-water supply. Since success or failure could depend on the persistence of the compounds in the various media, experiments were set up to evaluate stability under different conditions. As a result of these investigations the use of chemosterilants has been made more effective, a better understanding of aziridine analysis has been gained, and the degradative pathway of at least one aziridinyl compound, tepa, has been demonstrated. Of particular interest is the high sensitivity of tepa and other aziridinyl compounds to even mildly acidic substrates.

Experimental

Materials. Aziridine Compounds.—These are identified in Table I and will be referred to in the text by their common names.

Tepa.—The solvent from an 85% methanolic solution of the chemical, obtained from Interchemical Corp., New York, N. Y., was removed under reduced pressure and the residue cooled to 0° for several hours. The crystals were triturated, washed with hexane, filtered, pressed dry with a rubber dam, and placed in a vacuum desiccator over phosphorus pentoxide and potassium hydroxide. All transfers of the chemical were made in a drybox because tepa is highly hygroscopic. Our product melted at 41–43° (lit.[3] m.p. 41°).

TABLE I

Common name	Chemical name	Other designations
Tepa	Tris-(1-aziridinyl)-phosphine oxide	APO; aphoxide
Apholate	2,2,4,4,6,6-Hexahydro-2,2,-4,4,6,6-hexakis-(1-aziridinyl)-1,3,5,2,4,6-triazatriphosphorine	APN
Metepa	Tris-(2-methyl-1-aziridinyl)-phosphine oxide	MAPO; methaphoxide
Tretamine	2,4,6-Tris-(1-aziridinyl)-*s*-triazine	TEM
Ethylenimine	Aziridine or ethylenimine	

Tretamine (Chemirad Corp., East Brunswick, N. J.).—This compound was crystallized twice from ethyl acetate. It melted at about 139° dec. if the melting point was taken rapidly.[3]

Apholate[4] (Squibb Institute for Medical Research, New Brunswick, N. J.), **Metepa,** and **Ethylenimine** (Interchemical Corp.) were used as received.

Buffers for Thin Layer Chromatography.[5]—The following buffer solutions (MacIlvaine's and Sörenson's) were used: pH 3.0, 790 ml. of 0.1 *M* citric acid diluted to 1.0 l. with 0.2 *M* sodium dihydrogen phosphate; pH 5.0, 480 ml. of 0.1 *M* citric acid diluted to 1.0 l. with 0.2 *M* sodium dihydrogen phosphate; pH 7.5, 165 ml. of *M*/15 potassium monohydrogen phosphate diluted to 1.0 l. with *M*/15 sodium dihydrogen phosphate.

Deuterated Buffers.—A saturated solution of potassium dihydrogen phosphate in deuterium oxide was brought to a desired pH by the addition of solid sodium hydroxide. The solution was evaporated to dryness *in vacuo* and a sufficient amount of deu-

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