Biologically Oriented Organic Sulfur Chemistry. VI. Uses of o-Carboxyphenyl o-Carboxybenzenethiolsulfonate with Thiols[1]

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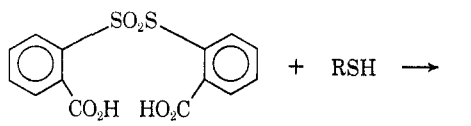
o-Carboxyphenyl *o*-carboxybenzenethiolsulfonate (**4**) was prepared easily in 77–92% yield from *o*-mercaptobenzoic acid in one step using chlorine or sulfuryl chloride. It reacted readily in ethanol or pH 7 buffer to give unsymmetrical *o*-carboxyphenyl disulfides (**5**–**15**), in yields exceeding 55%, with primary, secondary, and tertiary alkanethiols, an arenethiol, a heterocyclic thiol, and with mercapto amino acids. Other routes to these disulfides were inferior. The formation of *o*-carboxyphenyl disulfides affords a promising means of purifying and characterizing thiols, since typical compounds differ considerably in melting point and tlc R_f value, can be titrated with standard base, and can resist disproportionation well. Furthermore, the thiol can be regenerated by reduction either with sodium borohydride or dithiothreitol under mild conditions or recovered as the disulfide by disproportionation of the unsymmetrical disulfide. The *o*-carboxyphenylthio moiety seems promising for latentiating pharmacologically active thiols; it also reversibly blocks the sulfhydryl groups of an enzyme, although thus far it shows no advantages over Ellman's reagent.

Earlier work, in a continuing study of disulfides,[2] suggested that the *o*-carboxyphenylthio moiety, *o*-$HO_2CC_6H_4S$– (**1**), is a promising latentiating group[3] for radioprotective thiols.[5,6] It also suggested that salts of unsymmetrical disulfides containing moiety **1** showed an interesting instability.[5] While looking further into these matters, we realized that moiety **1** had attractive potentialities for latentiation of other medicinally significant thiols, for purification, characterization, or resolution of thiols, and for working with biochemically important thiol groups such as those of proteins. Relevant to many of these purposes were the presence of the carboxyl group and the probability of easy removal of moiety **1**. This paper reports a study of some of these potentialities.

We found earlier that *o*-mercaptobenzoic acid (**2**)[6] or its disulfide (**3**)[5] could be converted by chlorine–acetic acid–water in the Douglass–Farah reaction[7] to the thiolsulfonate (**4**). The **4** reacted with aminothiols to give compounds like **5**–**15** (eq 1).[5,6] With thiophenol as RSH, instead of an aminothiol, the reaction of eq 1 still proceeded cleanly, without added base at room temperature. As Table I shows, *o*-carboxyphenyl phenyl disulfide (**5**) precipitated in 85% yield. One recrystallization gave **5** of analytical purity in 74% yield.

Attempts to prepare **5** by treating *o*-carboxybenzenesulfenyl chloride (prepared from **2** with chlorine) with thiophenol gave **5** in 48% yield; *o*-carboxybenzenesulfenyl thiocyanate with thiophenol gave only impure **5** in 36% yield, at best.[8] In work by others, *o*-carboxybenzenesulfenyl chloride was prepared from *N*-chlorosuccinimide and thiol **2** and then was used to prepare an unsymmetrical disulfide;[9] in our hands this overall technique for the synthesis of **5** gave only symmetrical disulfides. Another approach was suggested by the well-known one for preparing disulfides by thioalkylating thiolates (RS^-) with thiosulfates (Bunte salts, $R'S\text{-}SO_3^-$);[10] unfortunately, the attempted preparation of the pyridinium thiosulfate needed for synthesis of **5** was unsuccessful, *i.e.*, of *o*-$HO_2CC_6H_4SSO_3^-C_5H_5NH^+$,[11] and this route was not explored further.



4

↑

(*o*-$HO_2CC_6H_4S)_2$

3

↑

2*o*-$HO_2CC_6H_4SH$

2

(1)

5, R = C_6H_5
6, R = C_2H_5
7, R = *n*-C_3H_7
8, R = *n*-C_4H_9
9, R = *n*-$C_{12}H_{25}$
10, R = *trans*-2-ClC_6H_{10}
11, R = $(CH_3)_3C$
12, R = 2-benzothiazolyl
13, R = L-$CH_2CH(NH_2)CO_2H$
14, R = D-$C(CH_3)_2CH(NH_2)CO_2H$
15, R = DL-$C(CH_3)_2CH(NHAc)CO_2H$

With eq 1 thus the synthesis of choice for **5** as a model, the best means for preparing **4** was sought. Improvement was made on chlorinolysis of the thiol **2**,[6] but use of liquid chlorine makes this technique inconvenient. Use of sulfuryl chloride instead of chlorine is much

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(1) (a) Paper V: L. Field, J. L. Vanhorne, and L. W. Cunningham, *J. Org. Chem.*, **35**, 3267 (1970). (b) This investigation was supported by Public Health Service Research Grant AM11685 from the National Institute of Arthritis and Metabolic Diseases. (c) Reported in part at the Southeastern Regional Meeting of the American Chemical Society, Tallahassee, Fla., Dec 1968, Abstracts, p 98, and at the IVth "Symposium on Organic Sulphur," Venice, Italy, June 1970. (d) Abstracted from the Ph.D. Dissertation of P. M. G., Vanderbilt University, May 1970. (e) Compounds of the structure *o*-$HO_2CC_6H_4SSR$ usually are named in this paper as unsymmetrical disulfides. The alternative (and more proper) naming as 2-(alkyl- or aryldithio)benzoic acids fails to emphasize the disulfide function, that of principal interest in this paper, and leads to problems in the consistent treatment of related compounds and groups to be discussed. (f) We are indebted to Professor J. P. Danehy of the University of Notre Dame for helpful comment.

(2) (a) For leading references, see paper XXIX in the series entitled "Organic Disulfides and Related Substances."[2b] (b) N. E. Heimer and L. Field, *J. Org. Chem.*, **35**, 3012 (1970).

(3) One which converts a biologically active compound to a derivative which *in vivo* either liberates the parent or allows an active moiety of it to react at a biologically important site. *Cf.* ref 4 for elaboration.

(4) L. Field, B. J. Sweetman, and M. Bellas, *J. Med. Chem.*, **12**, 624 (1969).

(5) R. R. Crenshaw and L. Field, *J. Org. Chem.*, **30**, 175 (1965).

(6) L. Field and H. K. Kim, *J. Med. Chem.*, **9**, 397 (1966).

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

(8) The procedure was based on one of H. Lecher and M. Wittwer, *Ber.*, **55B**, 1474 (1922).

(9) J. Tulecki, J. Dabrowski, and J. Kalinowska-Torz, *Diss. Pharm. Pharmacol.*, **18**, 473 (1966) [through *Chem. Abstr.*, **67**, 63971 (1967)].

(10) *Cf.* H. B. Footner and S. Smiles, *J. Chem. Soc.*, **127**, 2887 (1925).

(11) *Cf.* the method used to prepare $C_6H_5SSO_3^-C_5H_5NH^+$ developed by P. Baumgarten, *Ber.*, **63**, 1330 (1930).

TABLE I.—SYNTHESIS OF *o*-CARBOXYPHENYL DISULFIDES, *o*-$HO_2CC_6H_4SSR$

Compd	R	Time, hr (procedure)[a]	Yield, % (mp, °C)[b]	Mp, pure, °C[c]	C		H		S		Tlc R_f[d]	Neut equiv	
					Calcd, %	Found, %	Calcd, %	Found, %	Calcd, %	Found, %		Calcd	Found[e]
5	C_6H_5	3 (A)	85 (190–195 dec)	197–198.5 dec	59.51	59.79	3.84	3.74	24.45	24.23	0.17	262	260
6	C_2H_5	3 (A)[f]	109 (112–116)	129–130	50.44	50.64	4.70	4.49	29.93	30.13	0.65[g]	214	214
7	*n*-C_3H_7	1 (A)[f]	93 (80–95 dec)	95–100 dec	52.60	52.74	5.30	5.26	28.09	27.84	0.74[g]	228	220
8	*n*-C_4H_9	1 (A)[f]	101 (75–80 dec)	89–90 dec	54.51	54.34	5.82	5.78	26.46	26.39	0.22	242	240
9	*n*-$C_{12}H_{25}$	3 (A)[f]	55 (89–92)	91–91.5	64.36	64.30	8.53	8.72	18.09	18.22	0.26	355	358
10	*trans*-2-ClC_6H_{10}	1.5 (A)[h]	97[i] (146–149)	155–156	51.56	51.62	4.99	5.13	21.18	21.36	0.37	303	303
11	$(CH_3)_3C$	1 (A)[f]	84 (156–159)	162–163	54.51	54.73	5.82	5.60	26.46	26.57	0.18	242	233
12	2-Benzothiazolyl	4 (A)	55[j] (172–175)	173–174	52.64	52.83	2.84	2.89	30.12	30.23	0.10	319	321
13	L-$CH_2CH(NH_2)CO_2H$[k]	3 (A)	70 (198–199 dec)	198–199 dec	43.94	43.91	4.06	4.38	23.46	23.29	...[l]	137	139[l]
		1 (B)	84[m] (198.5–199 dec)										
14	D-$C(CH_3)_2CH(NH_2)CO_2H \cdot {}^1/_2H_2O$[n]	18 (A)	69[o] (155–159 dec)	163–164 dec[p]	46.43	46.67	5.20	5.34	20.66	20.73	0.58[p]	155	146[l]
		1 (B)	36[r] (158–160 dec)										
15	DL-$C(CH_3)_2CH(NHAc)CO_2H$	1 (A)	87 (208–211)										
		1 (B)	96 (200–205 dec)	208–209 dec	48.96	48.92	4.99	5.07	18.67	18.79	0.29[p]	172	171

[a] Hours allowed for reaction; for procedures A and B, see Experimental Section. [b] Yield of the disulfides with the melting point reported, calculated on the basis of eq 1. [c] Melting point of analytically pure disulfide; recrystallized from aqueous EtOH or MeOH except where noted. [d] Eastman chromagram sheet was used, Type 6060 (silica gel); after development with 9:1 $CHCl_3$–EtOH, the sheet was exposed to I_2 vapor. [e] In 95% EtOH (25 ml), titrated to a phenolphthalein end point with standard 0.1 *N* NaOH (no blank was necessary). The value reported is an average with three samples. [f] Addition of water (50 ml) and cooling required to initiate precipitation. [g] The chromagram sheet was let stand for 10 min in $CHCl_3$ saturated with HCl prior to use. [h] Reaction mixture evaporated to dryness and residue recrystallized from EtOH–H_2O below 25°. [i] After one recrystallization from EtOH–H_2O below 25°. [j] Cysteine hydrochloride dihydrate was used, but **13** precipitated; see text. Recrystallization from H_2O (100°) did not alter the properties of **13**. [k] Sparing solubility led to meaningless results. [l] Formol technique; see Experimental Section. [m] After crude product had been washed with acetone (100 ml). [n] Ether (800 ml) added to initiate precipitation. [o] Recrystallized from H_2O (100°). [p] Brinkman MN Polygram (polyamide) was used, developed with MeOH containing 0.5 %HCO_2H; spots observed under uv light. [q] Calcd for ${}^1/_2H_2O$: 2.94%. Found: 4.26%, probably because of slight hygroscopicity. [r] Clear reaction solution concentrated to 0.25 vol to induce precipitation.

more convenient;[12] the yield of about 92% compares favorably with about 91% obtained using chlorine.

Typical classes of *o*-carboxyphenyl disulfides (**5–15**), shown in eq 1 and Table I, then were prepared in 95% ethanol without base (procedure A), or in ethanol–buffer (procedure B). In most instances procedure A was preferred. With compounds **13–15**, Table I shows that procedure B is not essential; it was included for biologically significant compounds with which it might be crucial. Although thiolsulfonate **4** is quite stable as a solid, it decomposes slowly in ethanol or buffer solution with precipitation of the disulfide **3**; this instability should be of little concern, ordinarily, since the low content of *o*-carboxyphenyl disulfide (**3**) in the products shows that the rate of thioarylation must compare favorably with that of decomposition of **4**.[13]

The reaction in eq 1 was general for a wide variety of types of thiols (eq 1 and Table I). Compounds **5–15** were obtained in crude yields of 55–100% and usually became virtually analytically pure after one recrystallization.

Primary aliphatic thiols such as ethanethiol, 1-propanethiol, 1-butanethiol, and even the long-chained 1-dodecanethiol gave disulfides with attractive properties (**6–9**, respectively; Table I). The nearly odorless disulfides formed quickly at room temperature. The three homologs **6–8** were prepared to test differences in melting points of homologs. The melts of **7** and **8** remained cloudy up to about 220°; for **7**, this fact and the broad melting range (Table I) were shown to be caused by disproportionation at about the melting point by isolating the symmetrical disulfides. The differences in melting points of these homologs suggest considerable promise in use of *o*-carboxyphenyl disulfides for characterization (*vide infra*). Sodium salts of disulfides **6** and **7** have been reported, but details were unavailable to us.[14]

Secondary and tertiary alkanethiols, *trans*-2-chlorocyclohexanethiol, and 2-methyl-2-propanethiol gave disulfides **10** and **11** without difficulty or indication of reduced rate; the products were easier to purify than some from the primary thiols; and there was no indication of disproportionation at the melting point.

Similarly, the heterocyclic thiol 2-mercaptobenzothiazole (**16**) gave the disulfide **12** without incident.

The mercapto amino acids L-cysteine, D-penicillamine, and DL-*N*-acetylpenicillamine gave disulfides **13–15**, although the synthesis of **13** had a surprising feature. The reaction of L-cysteine hydrochloride with **4** should have led to the hydrochloride of **13**. Instead, very sparingly soluble material precipitated from either 95% ethanol or buffer–ethanol that contained no halogen. Elemental analysis and neutralization equivalent (formol technique)[15] indicated that **13** is the correct structure. For insight into this unusual precipitation of a free base from its hydrochloride, the hydrochloride

(12) A method developed by J. D. Buckman, M. Bellas, H. K. Kim, and L. Field, *J. Org. Chem.*, **32**, 1626 (1967).

(13) As a measure of the decomposition of **4**, the sparingly soluble disulfide **3** was separated which precipitated under the usual reaction conditions, except for absence of thiol. In 95% ethanol the results for time in hours (% decomposition) were 0.1 (<1), 1 (10), 8 (60), and 24 (94). In 5:2 ethanol–pH7 phosphate buffer, the results were: 0.5 (24), 1 (51), 2 (68), and 10 (96).

(14) H. Sasaki, *Igaku Kenkyu*, **27**, 2679 (1957) [through indexes to *Chem. Abstr.*, **52**, 11266 (1958); the compounds are not mentioned in the abstract itself].

(15) R. H. A. Plimmer, "Practical Organic and Bio-chemistry," Longmans, Green and Co., London, 1918, p 145 (*cf.* Experimental Section).

of **13** was prepared by dissolving **13** in *dry* methanol containing dry hydrogen chloride and evaporating the solvent (**13** itself is insoluble in dry methanol alone). When this product was shaken with water **13** precipitated, and titration with alkali showed that 103% of the theoretical amount of hydrochloric acid remained in the water. The extremely low solubility of **13** may be a factor in this precipitation from its salt. Disulfides **14** and **15** behaved as one would expect.

Latentiation of mercaptoethylamine with moiety **1** led to *o*-(2-protoaminoethyldithio)benzoate (**17**),[16] which gave "good" protection against radiation;[5,16b] **17** has since shown activity as an antiinflammatory drug.[17] Disulfides **13–15** will be tested as antiradiation and antiinflammatory drugs, but penicillamine is of special interest to us because of its use in rheumatoid arthritis; unfortunately, its use often leads to problems of toxicity.[20] Latentiation with the moiety **1** to give **14** might lead to a more active or less toxic counterpart. The acetyl derivative **15** had no effect on the skin-tensile strength of rats,[21] but acetylpenicillamine does not affect collagen in the skin of rats.[22a] Disulfide **14** had virtually the same effect in reducing skin tensile strength (54–59% of the tensile strength of a control) as penicillamine (54%).[22b]

For assurance as to the structures of **5–15**, a typical disulfide was synthesized independently. The reaction of *o*-mercaptobenzoic acid (**2**) with ethyl ethanethiolsulfonate gave **6** in 83% yield (eq 2). Further structural

$$\underset{\mathbf{2}}{o\text{-}HO_2CC_6H_4SH} + EtSO_2SEt \longrightarrow \underset{\mathbf{6}}{o\text{-}HO_2CC_6H_4SSEt} + EtSO_2H \quad (2)$$

evidence was provided by neutralization equivalents (Table I) and ir spectra. In common with past experience,[23] the ir spectra are not merely summations of those of the two symmetrical disulfides, although they usually resemble a summation. Not only are some bands in the symmetrical disulfides absent in the unsymmetrical one, but bands present in neither symmetrical one appear. The most characteristic ir bands are at 1670, 1300–1250, 900, 740, 690, and 650 cm^{-1}, although in **13–15** some were shifted somewhat. All of the disulfides were too sparingly soluble for good nmr spectra.

Disproportionation conceivably could become troublesome with disulfides resembling **5–15** (eq 3). Fortunately, disproportionation usually can be recognized easily in four ways. (1) The disulfide **3** is so sparingly soluble that it generally remains as a readily recognizable residue upon recrystallization (a feature which aids purification, as does the fact that RSSR usually either fails to dissolve or remains in solution if it does dissolve). (2) The other disulfide (RSSR), unless it has a carboxyl group, will be evident when the unsymmetrical one is dissolved in dilute aqueous base, as when the neutralization equivalent is determined. (3) Melting point depression usually is clear if there is contamination by either symmetrical disulfide. (4) Tlc results in **3** left at the origin if much **3** is present. Thus, with **10** and **11** presence of as little as 1% of **3** is apparent by tlc. None of the disulfides **5–15** showed presence of **3** by tlc after one or two recrystallizations, and all gave single spots except **13**, which was too sparingly soluble for tlc (Table I); in eight instances, disulfides corresponding to RSSR were done simultaneously and were reasonably distant from the tlc spot reported in Table I. Some tendency to disproportionate in tlc was observed with **6** and **7** (9:1 chloroform–ethanol; three spots were seen); this behavior was circumvented by prior washing of the tlc sheet with chloroform containing dry hydrogen chloride.

$$\underset{\mathbf{3}}{2o\text{-}HO_2CC_6H_4SSR} \longrightarrow (o\text{-}HO_2CC_6H_4S)_2 + RSSR \quad (3)$$

Under most circumstances, the *o*-carboxyphenyl disulfides studied resist disproportionation in solution well. For example, **5** was recovered in 96–98% yield after 21–119 hr at 100° in H_2O, 1,4-dioxane, or AcOH; that it then contained negligible **3** was shown by tlc and melting point.

The sodium salt of **5** in water, on the other hand, disproportionated completely in 1 hr at 68° and to the extent of 13% in 20 hr at about 25° (25% in ambient light). This unusual behavior of the *salt* is more a virtue than a blemish. It furnishes a useful means for recovery of a thiol as its symmetrical disulfide. Thus **5** gave phenyl disulfide in 96% yield after 5 min with *ca.* 12 equiv (to accelerate disproportionation) of aqueous alkali at *ca.* 25°; the symmetrical disulfide **3** was recovered in 98% yield by acidification. Although aryl disulfides undergo decomposition to sulfinic acids and thiols in the presence of base,[24] no such behavior was apparent with **5**, in conformity with observations that the disulfide **3** is by far the least reactive of the dithiodibenzoic acids in alkaline solution.[25] Symmetrical disulfides often afford a convenient means of storing thiols; they can be used in a wide variety of reactions without reconversion to the thiol.

Although conversion of an *o*-carboxyphenylthio derivative to the symmetrical disulfide should prove useful, regeneration of the thiol by reduction should be more so. In establishing the feasibility of mild reductions, the benzothiazolyl disulfide **12** was used as a model because 2-mercaptobenzothiazole (**16**) is easily isolated. Sodium borohydride (**18**), which has been used for reduction of disulfides,[26] reduced **12** in either 1,4-dioxane or dilute aqueous alkali to the thiol **16** in 82–85% yield (eq 4).

$$\underset{\mathbf{12}}{o\text{-}HO_2CC_6H_4SSC_7H_4NS} \xrightarrow[\text{DTT (19)}]{NaBH_4\ (18)\ \text{or}} \underset{\mathbf{16}}{HSC_7H_4NS} + \mathbf{2} \quad (4)$$

(16) (a) Nomenclature suggested by F. Y. Wiselogle. See F. G. Bordwell, M. L. Peterson, and C. S. Rondestvedt, Jr., *J. Amer. Chem. Soc.*, **76**, 3945 (1954). (b) *Cf.* L. Field and P. M. Giles, Jr., *J. Med. Chem.*, **13**, 317 (1970).

(17) We are indebted to Drs. N. G. Brink and C. G. Van Arman of the Merck Sharp and Dohme Research Laboratories for the following unpublished results: Carrageenin foot-edema assay,[18] 10 mg/kg (32%), 30 mg/kg (40%); adjuvant arthritis,[19] inactive at 50 mg/kg.

(18) Procedure of C. A. Winter, E. A. Risley, and G. W. Nuss, *Proc. Soc. Exp. Biol. Med.*, **111**, 544 (1962); responses to standard agents are reported.

(19) Procedure of H. C. Stoerk, T. C. Bielinski, and T. Budzilovich, *Amer. J. Pathol.*, **30**, 616 (1954).

(20) I. A. Jaffe, *Arthritis Rheum.*, **8**, 1064 (1965).

(21) We are indebted to Dr. I. A. Jaffe, of the New York Medical College and Flower and Fifth Avenue Hospitals (New York, N. Y.), for these tests using means referred to previously.[4]

(22) (a) M. E. Nimni, K. Deshmukh, N. Gerth, and L. A. Bavetta, *Biochem. Pharmacol.*, **18**, 707 (1969); (b) I. A. Jaffe, P. Merryman, and D. Jacobus, *Science*, **161**, 1016 (1968).

(23) *Cf.* ref 6.

(24) R. Schiller and R. Otto, *Ber.*, **9**, 1637 (1876).

(25) (a) S. Smiles and J. Stewart, *J. Chem. Soc.*, **119**, 1792 (1921); (b) S. Smiles and D. C. Harrison, *ibid.*, **121**, 2022 (1922); (c) J. P. Danehy and K. N. Parameswaran, *J. Org. Chem.*, **33**, 568 (1968).

(26) (a) C. R. Stahl and S. Siggia, *Anal. Chem.*, **29**, 154 (1957); (b) W. D. Brown, *Biochim. Biophys. Acta*, **44**, 365 (1960).

The *o*-mercaptobenzoic acid (**2**) also formed was removed easily by washing with aqueous sodium bicarbonate. Dithiothreitol (**19**, Cleland's reagent, DTT) also is a mild elegant reductant,[27] a particularly popular one for sensitive systems. One molar proportion of **19** reduced one of **12** to the thiol **16** in 35% yield. Since the reduction by **19** presumably is an equilibrium process, use of a larger excess of **19** (which is costly, however) should increase the yield of **16**; this trend is reflected by an increase in the yield of **16** to 66% with use of 2 molar proportions of **19**.

The ease of preparing, purifying and characterizing *o*-carboxyphenyl disulfides, then of conversion to a desired disulfide or thiol, suggests several uses other than latentiation. One use is for the characterization of thiols for which few good means are available. Some common derivatives, such as mercuric mercaptides, nitro thiol esters, α-anthraquinone sulfides, *p*-nitrophenyl sulfones, and 2,4-dinitrophenyl sulfides or sulfones, often exhibit unsatisfactory melting points or have a small range of melting points,[28a] although *N*,*N*-diphenylthiocarbamates seem promising.[28b] Some are not applicable to thiols which contain other functional groups. *o*-Carboxyphenyl disulfides generally not only lack these shortcomings but have several features which further enhance their value (*cf.* Table I). (1) They are easy to prepare and purify from **4**, a cheap, readily accessible reagent. (2) The carboxyl group permits determination of the neutralization equivalent and is valuable for isolation and purification. (3) The tlc R_f values vary considerably. (4) The melting points fall in a desirable temperature range, are usually rather well separated and differ even for homologs (*i.e.*, **6**–**8**). (5) A thiol can be either regenerated or recovered directly as its disulfide.

Another attractive use (not verified experimentally) would seem to be in the resolution of thiols. Conversion of a thiol to its disulfide by eq 1 could be followed by resolution with optically active amines, regeneration of the *o*-carboxyphenyl disulfide from its salt, and conversion of the unsymmetrical disulfide to the optical active thiol or disulfide.

A third possible application was attractive, that of using eq 1 for analysis or reversible "blocking" of the sulfhydryl (–SH) moieties of proteins.[29] Creatine kinase, an enzyme, after reaction with **4** showed total loss of enzymatic activity, indicating conversion of –SH to disulfide. No distinctive uv absorption useful for analysis of –SH was observed. Ellman's reagent reacted with creatine kinase more rapidly;[30] the reaction could be followed by uv absorption. Reduction of either inactivated product with excess **19** gave a 95–100% recovery of enzymatic activity, indicating regeneration of –SH. The thiolsulfonate **4** seems rather unpromising for assay of –SH and seems to have no advantage over Ellman's reagent for reversible blocking, unless its slower reaction could provide greater selectivity for different –SH groups, or unless its lack of color makes it useful in masking SH where the color from Ellman's reagent might interfere with other colored reagents.

(27) W. W. Cleland, *Biochemistry*, **3**, 480 (1964).

(28) (a) E. E. Reid, "Organic Chemistry of Bivalent Sulfur," Vol. I, Chemical Publishing Co., New York, N. Y., 1958, p 162; (b) R. G. Hiskey, F. I. Carroll, R. F. Smith, and R. T. Corbett, *J. Org. Chem.*, **26**, 4756 (1961).

(29) We are much indebted to Dr. L. W. Cunningham and Dr. C. S. Brown for these unpublished results and for their gracious permission to summarize them here. The Ph.D. Dissertation of C. S. B., Vanderbilt University, Jan 1970, may be consulted for details.

(30) 5,5′-Dithiobis(2-nitrobenzoic acid); see G. L. Ellman, *Arch. Biochem. Biophys.*, **82**, 70 (1959).

Experimental Section[31]

Materials.—*o*-Mercaptobenzoic acid (**2**, Aldrich Chemical Co.) was dissolved in 95% EtOH at *ca.* 60°, treated with decolorizing carbon, and filtered while hot. Water was added to incipient turbidity. Cooling and filtration gave fine yellow crystals having mp 165–166° (lit.[32] mp 163–164°). *trans*-2-Chlorocyclohexanethiol[33] had bp 95° (30 mm) and n^{26}D 1.4986 [lit.[33a] bp 83° (20 mm); n^{20}D 1.5015]. Ethyl ethanethiolsulfonate was kindly provided by Dr. Michael Bellas.[12] All other reagents were used as purchased.

Preparation of *o*-Carboxyphenyl *o*-Carboxybenzenethiolsulfonate (4). A. Using Cl_2.—In an improvement of earlier procedures,[5,6] Cl_2 (20.9 g, 13.5 ml, ~0.3 mol) was condensed and then introduced slowly (1 hr) into a stirred mixture of the thiol **2** (30.2 g, 0.196 mol) and AcOH (5.6 ml, ~0.1 mol) in CH_2Cl_2 (110 ml) at −2° to +2°. Moisture was carefully excluded from the system by means of drying tubes containing $CaCl_2$. The suspension was stirred (20 min) at −2° to +2°, after which H_2O (3.6 ml, 0.2 mol) was added slowly (15 min). After 16 hr at *ca.* 25°, crude **4** was separated by filtration, washed with cold H_2O (200 ml), and dried under reduced pressure: yield of **4**, 30.2 g (91%); mp 200–210° dec. The **4** was taken up in 95% EtOH (*ca.* 1 g/10 ml) at *ca.* 25° and filtered. Water was added to incipient turbidity. Cooling (*ca.* 0°) and filtration gave **4** as a tan powder (25.6 g, 77%), mp 220–225° dec, unchanged by further crystallization (lit. mp 218–222° dec,[5] 215–223° dec).[6]

Anal. Calcd for $C_{14}H_{10}O_5S_2$: C, 49.69; H, 2.98; S, 18.95. Found: C, 49.58; H, 3.37; S, 18.86.

Thin layer chromatography of compound **4** on Brinkmann MN Polygram Sheet (polyamide) developed with MeOH containing 0.5% HCO_2H showed only one spot under uv light (R_f 0.20). The **4** thus obtained was identical (ir) with authentic **4**.[5] A sample of **4** was unchanged (ir, mp) after 5 years.

B. Using SO_2Cl_2.—In a procedure resembling one reported,[12] SO_2Cl_2 (81.00 g, 0.60 mol) was added slowly (1.5 hr) to a rapidly stirred solution of the thiol **2** (61.68 g, 0.40 mol) and AcOH (12.00 g, 0.20 mol) in CH_2Cl_2 (400 ml) at −2° to +2°. The reaction mixture was allowed to warm (during *ca.* 1 hr) to *ca.* 25°. Heating (40°, 3 hr) then resulted in evolution of copious amounts of gas. Water (7.30 g, 0.41 mol) was added slowly (0.5 hr) and heating was continued for 5 hr more. Tan solid was separated, washed first with cold H_2O (400 ml) and then with cold CH_2Cl_2 (100 ml), and then dried to give **4**, 62.38 g (92%), mp 210–216° dec. Thiolsulfonate **4** at this point was nearly always satisfactory for preparing **5**–**15**, even though the melting point was occasionally 200–220° dec. Unsuitability, suggesting the recrystallization described next, was determined easily by a significant residue of **3** when the 10% solution of **4** used for such preparations was prepared in EtOH. Recrystallization of a 10-g sample from aqueous EtOH as above gave 8.4 g (84%) of **4** having mp and mmp 220–224° dec, identical (ir) with authentic **4**.[5]

Longer reaction times without heating gave **4** in only 30% yield, mp 220–222° dec.

Synthesis of *o*-Carboxyphenyl Disulfides 5–15 *via* Eq 1.—Except for variations noted in Table I, procedures A and B were as illustrated below.

Procedure A. *o*-Carboxyphenyl Phenyl Disulfide (5).—Thiophenol (1.65 g, 15 mmol, often in a little EtOH) was added to a stirred solution of **4** (5.06 g, 15 mmol) in 95% EtOH (50 ml). Precipitation of **5** began in *ca.* 5 min. Filtration after 3 hr gave **5** (3.00 g, 76%), mp 190–195° dec. A second crop was collected (0.36 g, 9%), mp 190–195° dec. The crude **5** (1 g) was dissolved in EtOH (50 ml) at *ca.* 25°, and water was added to incipient

(31) Melting points are corrected. Elemental analyses were by Galbraith Microanalytical Laboratories, Knoxville, Tenn. Ir spectra were done using a Beckman Model IR-10 with KBr pellets of all samples; s signifies a strong absorption band (others reported were medium). Unless otherwise stated, reactions were carried out at room temperature. Solvents were removed under reduced pressure with a rotary evaporator.

(32) C. F. H. Allen and D. D. MacKay, "Organic Syntheses," Coll. Vol. II, Wiley, New York, N. Y., 1943, p 580.

(33) (a) Prepared according to C. C. J. Culvenor, W. Davies, and N. S. Heath, *J. Chem. Soc.*, 282 (1949); (b) E. E. van Tamelen, *J. Amer. Chem. Soc.*, **73**, 3444 (1951), reported that the product obtained is the trans isomer.

turbidity. Cooling and filtration gave 0.87 g (87% recovery, 74% yield) of **5** as fine white crystals having mp 197–198.5° dec; ir (KBr) 3200–2500, 1670 (s), 1420, 1320, 1280, 1260, 900, 740, 690, and 650 cm^{-1}.

D-Penicillamine did not dissolve quickly in the reaction medium, but finely ground penicillamine did so during a long reaction period of 18 hr. Ether then was added to the clear solution to effect precipitation. Disulfide **14** had $[\alpha]^{25}D$ +181° (*c* 1 in glacial acetic acid).

Procedure B. DL-*o*-(**1,1-Dimethyl-2-carboxy-2-acetamidoethyldithio)benzoic Acid (15).**—DL-*N*-Acetylpenicillamine (1.91 g, 10 mmol) was suspended in commercial phosphate buffer (pH 7, 0.2 *M*, 20 ml), and the mixture was added to a stirred solution of **4** (3.38 g, 10 mmol) in 95% EtOH (50 ml). After 1 hr, the heterogeneous reaction mixture was cooled, and the **15** was removed by filtration, yield 3.28 g (96%), mp 200–205° dec. Recrystallization from MeOH–H_2O gave material having mp 208–209° dec; ir (KBr) 3360, 3200–2300, 1695 (s), 1625, 1540, 1250 (s), 900, and 740 cm^{-1}.

The formol titration technique[15] is illustrated for disulfide **13**. Commercial formalin (10 ml) was diluted with H_2O (20 ml) and neutralized with 0.1 *N* NaOH using phenolphthalein as an indicator. This neutral formalin was added to a suspension of **13** (0.2133 g, 0.78 mmol) in 95% EtOH (10 ml). Titration with NaOH (0.0994 *N*, 15.40 ml) indicated a neutralization equivalent of 139 (calcd, 137).

Comparative Syntheses of 5 by Other Means. A. *Via* **o-Carboxybenzenesulfenyl Chloride.**—Chlorine (7.09 g, 4.58 ml, 0.10 mol) was condensed (Dry Ice) and then was added slowly (0.5 hr) to a stirred suspension of **2** (15.42 g, 0.10 mol) in CH_2Cl_2 (55 ml) at *ca.* 0°. After 10 min more at 0°, PhSH (11.01 g, 0.10 mol) was added slowly (15 min). The reaction mixture was allowed to warm to *ca.* 25°, and the solvent was removed under reduced pressure. Recrystallization of crude **5** from EtOH–H_2O as before gave **5** (12.5 g, 48%) with mp 190–198° dec, identical with authentic **5** by ir.

B. *Via* **o-Carboxybenzenesulfenyl Thiocyanate** (*Cf.* **Ref 8**).—The thiol **2** (1.54 g, 10 mmol) in Et_2O (50 ml) was added slowly (1 hr) to a stirred solution of $(SCN)_2$ (1.33 g, 11 mmol) in Et_2O (55 ml) at *ca.* 0°. After 1 hr, PhSH (1.10 g, 10 mmol) in Et_2O (10 ml) was added rapidly. The solution was stirred for 10 min more at *ca.* 0° and for 3 hr at *ca.* 25°. The Et_2O solution was washed with H_2O until colorless and then dried ($MgSO_4$). The Et_2O was removed to give crude **5**; the ir was similar to (but not identical with) that of authentic **5**. Recrystallization as usual gave 0.95 g (36%) of **5** having a broad melting point of 145–180° dec. It might be added that, although the use of excess $(SCN)_2$ is standard,[8] use of smaller amounts might be advantageous here.

Independent Synthesis of o-Carboxyphenyl Ethyl Disulfide (6).—Ethyl ethanethiolsulfonate (1.54 g, 10 mmol) and **2** (1.54 g, 10 mmol) were stirred (1 hr) at *ca.* 25° in 95% EtOH (50 ml). Addition of H_2O (50 ml) and cooling gave 1.78 g (83%) of **6**, mp 125–128°. Recrystallization from MeOH–H_2O as usual gave 1.60 g (75%) of **6**, mp and mmp 129–130°, ir identical with that of **6** synthesized by procedure A.

Reactions of o-Carboxyphenyl Disulfides. A. Disproportionation.—Carefully weighed samples of **5** (*ca.* 1 mmol) were dissolved in 10 ml of solvent (see Table II) in 15-ml ampoules. The ampoules were wrapped with aluminum foil to protect the contents from light. They were immersed to their necks in an oil bath and were maintained at 25, 68, or 100° for the designated time intervals. The ampoules then were withdrawn and chilled in ice. The extent of disproportionation was determined either by isolating phenyl disulfide which was formed, or by recovering disulfide **5**. The phenyl disulfide, isolated by filtration from all experiments involving the sodium salt of **5** in H_2O, was washed with H_2O (100 ml) and carefully dried (identity established by melting point and tlc). In all other instances the solvent was removed under reduced pressure and phenyl disulfide was separated by washing the residue with hexane (100 ml). The recovered **5** (hexane insoluble) was identical with authentic material (ir, melting point). "Disproportionation, %" was calculated as usual;[34] the results are given in Table II.

TABLE II

DISPROPORTIONATION OF **5** OR OF ITS SALT

Time, hr	Temp, °C	Solvent	%[a]
20	25	H_2O[b]	13
25	25	H_2O[b]	25[c]
79	25	H_2O[b]	69
0.25	68	H_2O[b]	42
0.75	68	H_2O[b]	83
1.00	68	H_2O[b]	100
0.1	25	H_2O[d]	96[e,f]
24	100	1,4-Dioxane	<3[f]
119	100	AcOH	<2[f]
21	100	H_2O[g]	<4[f]
24	25	95% EtOH	0[f]

[a] Disproportionation, %; see ref 34. [b] Containing 1 molar proportion of NaOH. [c] Exposed to ambient light. [d] Containing 12 *M* proportions of NaOH. [e] 98% of the theoretical amount of disulfide **3** also was recovered. [f] Determined by isolation of disulfide **5**. [g] Sample did not dissolve even at 100°. The **5** was completely dissolved in all the other experiments reported in Table II.

B. Reduction with $NaBH_4$ (18).—A solution of **18** (0.67 g, 17.70 mmol) and disulfide **12** (1.14 g, 3.57 mmol) in dry 1,4-dioxane (50 ml) was warmed on a steam bath for 3 hr. The solution then was acidified carefully (pH 1, 10% HCl). After filtration to remove solids, the solvent was removed under reduced pressure. The solid residue was rubbed with 5% $NaHCO_3$ (100 ml) and H_2O (50 ml) and then dried to give 0.49 g (82%) of yellow crystalline **16**, mp and mmp 180–181°, ir identical with that of authentic **16**.

A similar reaction using 0.1 *N* aqueous NaOH as the solvent gave **16** in 85% yield, identified by melting point and ir.

C. Reduction with Dithiothreitol (19).—A solution of disulfide **12** (0.32 g, 1.0 mmol) and **19** (0.31 g, 2.0 mmol) in pH 10 buffer (0.2 *M* carbonate, 20 ml) was stirred for 20 min. Acidification to pH 1 (concentrated HCl) and cooling gave yellow **16**, which was separated and washed with 5% $NaHCO_3$ (100 ml) and H_2O (50 ml). The residue of crude **16** (0.16 g, 96%; mp 160–165°) was recrystallized from EtOH–H_2O to give 0.11 g (66%) of **16**, identified by ir, mp and mmp 180–181°.

When 1.0 mmol of **19** was used, 0.06 g (35%) of **16** was recovered, identified by melting point and ir.

Registry No.—**4**, 1906-41-8; **5**, 26929-62-4; **6**, 26929-63-5; **7**, 26893-47-0; **8**, 26893-48-1; **9**, 26893-49-2; **10**, 26885-61-0; **11**, 26893-50-5; **12**, 26893-51-6; **13**, 26885-62-1; **14**, 26885-63-2; **15**, 26885-64-3.

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