Biologically Oriented Organic Sulfur Chemistry. 9. Carbonyl and Thiocarbamoyl Disulfides as Inhibitory Agents for *Histoplasma capsulatum*[1a-d]

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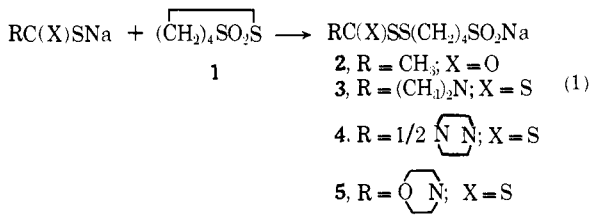
Dithiocarbamates and thiuram disulfides have long been noted for antifungal activity against human pathogens.[2] More specifically, potassium *N*,*N*-dimethyldithiocarbamate inhibited *Histoplasma capsulatum* at <10 μg/ml *in vitro*, and some trithiopercarbamates, $R^1R^2NC(S)SSR^3$, at 1–5 μg/ml.[3] Thioacetic and thiopropionic acid also inhibited *H. capsulatum* (10–20 μg/ml).[4] Latentiation of thioacetic and other thio acids as the carbonyl disulfides, $R^1C(O)SSR^2$, produced compds having *in vitro* activity at concns ranging upward from 8 μg/ml, suggesting that R^2S moieties without functional groups were not particularly promising for latentiation.[5] Since the moiety $S(CH_2)_4SO_2Na$ gave quite good results when used to latentiate a radioprotective thiol, 2-acetamidoethanethiol,[6] this moiety seemed a worthwhile one to try with dithiocarbamates and a thio acid.

Compds **2**–**5** (Table I) were prepared by oxodisulfide cleavage,[6] in which the appropriate thiolate reacts with 1,2-dithiane 1,1-dioxide (**1**), as shown by eq 1; these reactions thus extend oxodisulfide cleavage to include thio and dithiocarbamic acid salts.



$$RC(X)SNa + \underbrace{(CH_2)_4SO_2S}_{1} \longrightarrow RC(X)SS(CH_2)_4SO_2Na \quad (1)$$

2, R = CH_3; X = O
3, R = $(CH_3)_2N$; X = S
4, R = 1/2 ; X = S
5, R = ; X = S

Abs EtOH, a solvent used earlier for oxodisulfide cleavage,[6] was satisfactory for the prepn of **4**. However, in the prepn of **2**, **3**, and **5**, its use led to products containing impurities that could not be removed. Abs Et_2O or $CHCl_3$ gave good results. The latter reactions were carried out heterogeneously, excess **1** being used to assure complete reaction of the salts. The ppt was separated, and **1** was removed by washing with Et_2O (**2**, **5**) or by pptn of **3** from MeOH with Et_2O; with **4**, the reaction was homogeneous throughout, and EtOH was simply evapd, after which **1** was removed using MeOH–Et_2O. The crude products were obtained in yields of 66–100%. Structures of **2**–**5** were confirmed by the ir spectra, through appearance of absorption at ~975 cm^{-1} (SO_2^-) and loss of absorptions at 1120 and 1310 cm^{-1} (SO_2), and by nmr spectra in D_2O through appropriate numbers and relationships of protons. The purity of disulfides **2**–**5** was assured by tlc in MeOH–$CHCl_3$ (2:1). In all instances the symmetrical disulfide, sodium 4,4′-dithiobis(butanesulfinate), could be clearly distinguished from **2**–**5** by tlc; also, $(AcS)_2$ and $[(CH_3)_2NC(S)S]_2$ were easily separated from **2** and **3**.

Compds **3** and **4** were purified by fractional repptn from MeOH–Et_2O.[6] Repptn was most successful when carried out rapidly at 0–10° to minimize contact with MeOH. It has been found that certain unsymmetrical disulfides containing the $(CH_2)_4SO_2Na$ moiety disproportionate rapidly in MeOH.[7]

The stabilities of **2**–**5** are noticeably different. Compds **3** and **4** are stable under ambient conditions for months, but **2** begins to decompose visibly after 24 hr at room temp (samples become black). Atmospheric moisture, O_2, and light are not responsible for the decomposition of **2**, since decomposition in darkened or N_2-purged ampules seems to proceed much as it does without these precautions (all samples became black at about the same rate). However, **2** could be kept unchanged at 0° for months under the latter conditions. The reason for the facile decomposition of **2** has not been determined. Compd **5** is very hygroscopic and must be stored under N_2 or in a vacuum desiccator. Under these conditions **5** is stable for months at 25°.

Two other compds in which X of eq 1 was S, and R was 4-acetyl-1-piperazinyl or 4-benzyl-1-piperazinyl, were prepared in crude yields of 72 and 50%, resp, by treating the reaction product of the piperazine, CS_2, and NaOH with **1**. Neither compd could be purified by repptn or otherwise. *In vitro* tests on the crude acetylpiperazinyl compd for inhibitory effects were unpromising (MIC > 20).

Table I shows the results of *in vitro* tests of **2**–**5** in comparison with amphotericin B.[8] Compds **2** and **4** were fairly active at best. The MIC of both **3** and **5**, however, was 2.5 and 1 μg/ml, resp, when tested against strains H-7 and H-25 of *H. capsulatum*. The MIC of potassium *N*,*N*-dimethyldithiocarbamate (**6**) was 10 and 2.5 μg/ml, resp, for H-7 and H-25. Hence, on a weight basis, 2.5–4 times more of **6** was required for inhibition than of the latentiated forms **3** and **5**. On a molar basis, the amount of **6** required for in-

(1) (a) Paper 8: B. J. Sweetman, M. M. Vestling, S. T. Ticaric, P. L. Kelly, L. Field, P. Merryman, and I. A. Jaffe, *J. Med. Chem.*, **14**, 868 (1971). (b) This investigation was supported by Public Health Service Research Grant No. AM11685 from the National Institute of Arthritis and Metabolic Diseases (L. F.) and (in early phases) by the U. S. Army Medical Research and Development Command, Department of the Army, under Research Contract No. DA-49-193-MD-2030 (L. F.), and also by Public Health Service Research Grant No. AI-08916 from the National Institute of Allergy and Infectious Diseases (I. McV.). (c) Taken from part of the Ph.D. Dissertation of W.S.H., Vanderbilt University, Nashville, Tenn., Jan 1971. (d) Reported in part at The Second National Conference on Histoplasmosis, Atlanta, Ga., Oct 6–8, 1969.

(2) G. D. Thorn and R. A. Ludwig, "The Dithiocarbamates and Related Compounds," Elsevier Publishing Co., New York, N. Y., 1962, p 207 ff.

(3) I. McVeigh and Z. Evans, *Mycopathol. Mycol. Appl.*, **35**, 313 (1968).

(4) I. McVeigh, Z. Evans, L. Field, and W. Hanley, *ibid.*, **37**, 349 (1969).

(5) (a) L. Field, W. S. Hanley, I. McVeigh, and Z. Evans, *J. Med. Chem.*, **14**, 202 (1971); (b) Latentiation refers to the conversion of a compd to a derivative that will liberate the parent compd enzymatically *in vivo*. In our view, this concept is useful for suggesting approaches to drug design, even though one has no assurance that any activity found *actually* is a result of the parent compd being liberated enzymatically. Indeed, activity could well result from the intact molecule, from the latentiating group itself, or from some other moiety of the molecule. For further discussion and references, *cf.* ref 5c; (c) L. Field, B. J. Sweetman, and M. Bellas, *J. Med. Chem.*, **12**, 624 (1969).

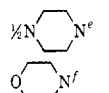
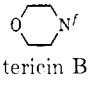
(6) L. Field and R. B. Barbee, *J. Org. Chem.*, **34**, 1792 (1969).

(7) Private communication from Y. H. Kim.

(8) Tested as described previously;[5a] we are indebted to Z. Evans for these tests.

TABLE I
SYNTHESIS AND *in Vitro* ACTIVITIES OF CARBONYL AND THIOCARBAMOYL DISULFIDES

$$RC(X)SNa + 1 \rightarrow RC(X)SS(CH_2)_4SO_2Na$$

Compd	$RC(X)SS(CH_2)_4SO_2Na$ R	X	React solvent	Product Yield, %	Product Mp, °C	Purified mp, °C	Formula	MIC, μg/ml[a] H-7	MIC, μg/ml[a] H-25	Anal.[b]
2	CH_3	O	Et_2O	100	101–103 dec[c]	101–103 dec[d]	$C_6H_{11}NaO_5S_3$	>20	10	C, H, Na, S
3	$(CH_3)_2N$	S	Et_2O	88	150–155	160–161	$C_7H_{14}NNaO_2S_4$	2.5	1.0	C, H, N, Na, S
4		S	EtOH	66	170 dec	180 dec	$C_{14}H_{24}N_2Na_2O_4S_8$	>20	10	C, H, N, Na, S
5		S	$CHCl_3$	79	140–142	140–142[d]	$C_9H_{16}NNaO_3S_4$	2.5	1.0	C, H, O, N, Na, S[g]
Amphotericin B								0.2[h]	0.1[h]	

[a] Minimum inhibitory concn. The highest concn tested was 20 μg/ml in H_2O. [b] Unless otherwise noted, where analyses are indicated only by symbols of the elements, anal. results for those elements were within ±0.4% of the theor values. [c] Detd by insertion at 95° and raising the temp 2°/min. The sample did not give a clear melt. The range of decompn was much broader when the sample was heated slowly from room temp. [d] The ether-washed sample was dried at 25° (0.01 mm) and analyzed. Repptn from MeOH caused decompn. [e] Stirred 1.5 hr at room temp. [f] Stirred 1 hr at room temp. [g] Difficulty and variability of analysis seem to be characteristic of **5**. The same sample was sent simultaneously to 3 different analytical laboratories (Galbraith Microanalytical Laboratories, Atlantic Microlab, Inc., and Eli Lilly and Co., the latter kindly being arranged by Dr. W. B. Lacefield and carried out by Mr. G. Maciak). The results were: calcd: C, 32.03; H, 4.78; O, 14.22; N, 4.15; Na, 6.81; S, 38.00. Found: C, 21.66, 30.97, 30.93, 30.86, 28.07; H, 4.67, 5.00, 5.87, 6.04, 6.34; O, 18.84, 21.60, 23.74; N, 3.83, 3.82, 3.81, 3.52, 3.31; Na, 6.92, 7.33, 5.98, 5.42; S, 37.78, 37.51, 36.68, 36.49, 34.18, 28.74. These results suggest that variable hydration may play a role in the difficulty of analysis. [h] *Cf.* ref 5a.

hibition of H-7 and H-25 was 5–7 times that of **3** and 5–8 times that of **5**. The molar ratios thus show a clearly beneficial effect on activity of the $S(CH_2)_4SO_2Na$ moiety. Actually, the activity of **5** is even more striking because *in vitro* tests of **7** showed it to be

6 **7**

inactive at 20 μg/ml. *In vivo* tests of **5** were done essentially as described earlier, by studying the prolongation of the life of mice that had been exposed to X-rays and then infected with *H. capsulatum* before treatment with **5**.[9] Five daily sc doses of **5** in amounts of 50, 25, and 12.5 mg/kg resulted, resp, in extension of survival above untreated controls (average survival, 10 mice, 5.1 ± 0.1 days) of 12, 14, and 18%; **5** was not toxic at these levels. Amphotericin B, given similarly at 12.5 mg/kg, effected extension of 31%. Low variations were encountered in the test, so that although the activity of **5** is weak it has high statistical significance.

As part of a continuing study of the protective effects of disulfides against otherwise lethal amounts of ionizing radiation, **3** was tested as an antiradiation drug.[10] Although certain other thiocarbamoyl disulfides had "good" activity as antiradiation drugs,[11] **3** afforded no protection at levels of 30 mg/kg in mice (LD_{50} 60 mg/kg). For an effective drug, R^3 in the structure $R^1R^2NC(S)SSR^3$ seemingly should be a tertiary rather than a primary group (*cf.* also ref 11).

(9) Tests, kindly arranged by Dr. W. B. Lacefield, were carried out under the supervision of Dr. R. S. Gordee of Eli Lilly and Company, Indianapolis, Ind., essentially as described in ref 5a; the evaluation system has now been described further by R. S. Gordee and T. R. Matthews, *Appl. Microbiol.*, **20**, 624 (1970).

(10) Tested as described previously,[5c] through the kindness of Drs. D. P. Jacobus, T. R. Sweeney, and E. A. Steck, and of Miss Marie Grenan of the Walter Reed Army Institute of Research, Washington, D. C.

(11) L. Field and J. D. Buckman, *J. Org. Chem.*, **33**, 3865 (1968).

Experimental Section[12]

Materials.—4-Acetylpiperazine·HCl[13] and **1**[14] were prepd by published procedures. Dithiocarbamates were prepd by the addn of CS_2 to the appropriate amine in DMF or H_2O; after a stirring period of 0.5 hr, an equiv amt of NaOH was added, and the soln was stirred an addnl 0.5 hr; products were isolated by solvent evapn and used without further purification. All other materials were used as purchased.

Sodium 4-(Acetyldithio)butanesulfinate(2).—Methanolic NaOMe (9 ml of 1.0 *M* soln) was added dropwise to **1** (5.97 g, 30.3 mmoles) and thioacetic acid (0.68 g, 95% SH, 8.5 mmoles) in 100 ml of abs Et_2O. The heterogeneous mixt was stirred for 5 min at room temp. The ppt was removed and washed with 125 ml of cold Et_2O and was dried to give 2.2 g (100%) of white **2**, mp 101–103°. Tlc (2:1 MeOH–$CHCl_3$) showed only one spot (R_f 0.60); ir 2910, 1740, 1460, 1385, 1110, 1010, 975, and 715 cm^{-1}; nmr, δ 1.6–2.4 (m, 4), 2.55 (s, 3), 2.6–3.1 (m, 2), and 3.1–3.6 (m, 2).

The same procedure was used for preparing **5**, except that the solvent used was $CHCl_3$.

Sodium 4-(*N*,*N*-Dimethylthiocarbamoyldithio)butanesulfinate (3).—Sodium dimethyldithiocarbamate (3.7 g, 26 mmoles) was added to a soln of **1** (7.9 g, 52 mmoles) in 50 ml of abs Et_2O and the heterogeneous mixt was stirred for 5 hr. The ppt was removed and amounted to 6.8 g (88%) of white **3**, mp 150–155°. Purification was effected by adding cold Et_2O to **3** dissolved in a min of MeOH at 0–10° until some ppt formed; the ppt was removed and more Et_2O was added to ppt a second fraction. This procedure was done 4 times. The first and last fraction amounted to ~20% of the initial product. The middle 2 fractions (~80%) were combined, and the procedure was repeated; 3 repetitions gave **3** with mp 160–161°; each required ~0.5 hr. Tlc showed only one spot (R_f 0.59); ir 3440, 3260, 2930, 2870, 1495, 1465, 1380, 1245, and 975 cm^{-1};[15] nmr δ 1.8–2.3 (m, 4), 2.5–2.8 (m, 2), 3.0–3.3 (m, 2), and 3.8 (s, 6).

The same procedure was used for preparing **4** except that EtOH was used as the solvent; the product was recovered by evapn of EtOH, and the **4** then was fractionally pptd.

(12) Melting points, determined in capillary tubes using a Mel-Temp hot-block apparatus, are corrected. Except where otherwise specified, elemental analyses were by Galbraith Microanalytical Laboratories, Knoxville, Tenn. Ir spectra were obtained using a Beckman Model IR 10 spectrophotometer with Nujol mulls of solids; bands reported are at least of medium intensity. Nmr spectra were obtained in D_2O using a Varian Model A-60 spectrometer (TMS external, HOD as internal check). Tlc usually was performed on Eastman Chromagram No. 6060 sheets (silica gel) using MeOH or MeOH–$CHCl_3$ (2:1), with visualization by I_2 vapor and with observation of spots after 5 min.

(13) R. Baltzly and E. Lorz, U. S. Patent 2,436,685 (1948); *Chem. Abstr.*, **42**, 4616 (1948).

(14) L. Field and R. B. Barbee, *J. Org. Chem.*, **34**, 36 (1969).

(15) The absorptions at 3440 and 3260 cm^{-1} seem anomalous. However, presence of an impurity seems improbable when one considers the method of synthesis, tlc, nmr, and analysis.