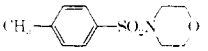
TABLE III

REACTION BETWEEN TRIFLUOROMETHYL *p*-TOLUENETHIOLSULFONATE AND SECONDARY AMINES

Amine	*p*-Toluenesulfonamide	Mp, °C	Yield, %
		146.5–148[a]	75
		298–300[b]	85

[a] D. Klamann and H. Bertsch, *Chem. Ber.*, **89**, 2007 (1956), give mp 148°. [b] Lit.[a] mp 296–298°.

bands appeared. Band 1 had R_f *ca.* 0.35, the usual thiolsulfonate position, and band 2 R_f *ca.* 0.8, usually associated with a disulfide. Band 1 was extracted with ether and a crystalline material melting at 90–95° was obtained. Band 2 gave mica-like flakes which melted at 89–90°. Since the two compounds melted so closely and since the previously described compound, *p*-$CH_3C_6H_4SO_2SC_6H_4Br$-*p*, was reported to melt at 107°,[12] it was important to prove that both *p*-bromophenyl *p*-toluenethiolsulfonate and bis(*p*-bromophenyl) disulfide were present. A comparison of the infrared spectrum of the material from band 2 with a spectrum of an authentic sample of bis(*p*-bromophenyl) disulfide, mp 89–91°, showed the two to be identical. The spectrum of the crystals from band 1 was identical with that of the recrystallized compound of mp 93.5–94.5°. With these data and confirmation by the elemental analysis it appears that the compound reported by London[12] may not have been *p*-bromophenyl *p*-toluenethiolsulfonate; total yield of thiolsulfonate (RSO_2SR') from this reaction, 2.3 g (67%).

In order to determine the fate of the SCF_3 group, the reaction between trifluoromethyl *p*-toluenethiolsulfonate and thiophenol was performed in a sealed system containing a gas cell. Trifluoromethyl *p*-toluenethiolsulfonate (2.56 g, 0.01 mole) was dissolved in 10 ml of absolute EtOH in a heavy-wall tube and frozen with liquid N_2. To this was added 1.1 g (0.01 mole) of thiophenol and the system was evacuated. The reaction tube was then allowed to warm to room temperature, the gas cell was removed, and spectra were determined.

The solution from the reaction tube was allowed to stand overnight, and a crystalline product which melted at 75–77° was separated. Chromatography of the remaining solution gave more material melting at 75–77° and another substance melting at 56–58°. The compound, phenyl *p*-toluenethiolsulfonate, has been reported as melting at 76–77°.[15] Commercial phenyl disulfide, mp 59–60°, and the disulfide isolated had identical infrared spectra. Total yield of phenyl *p*-toluenethiolsulfonate was 1.13 g (43%).

B. Reactions with Amines (Table III).—A solution of 0.87 g (0.01 mole) of morpholine in 10 ml of absolute ether was treated with 1.28 g (0.005 mole) of crude trifluoromethyl *p*-toluenethiolsulfonate in 10 ml of absolute ether. (The 100% molar excess of morpholine was not necessary.) The reaction was immediate and exothermic and caused some of the ether to boil away. The remaining ether was removed by moderate heat and the gelatinous mass was taken up in warm 2-propanol, then cooled at 4° for several hours. From the solution 0.9 g (75%) of white crystals were recovered, mp 144°. Two more recrystallations from 2-propanol gave a compound which had strong SO_2 absorption and was identified as *p*-toluenesulfonmorpholide.

A similar experiment using piperazine in absolute EtOH produced a white crystalline material; SO_2 absorption, identified as bis-*p*-toluenesulfonpiperazide.

Antibacterial Testing.—Bactericidal activity as the minimum lethal concentration (MLC) to *Staphylococcus aureus* was determined by the broth dilution method.[16] Trypticase soy broth made up to one-eighth the normal strength was employed to make serial dilutions of the compounds. This lower strength broth still produced heavy growth of the bacteria in 24 hr at 37° but presented less opportunity for undesirable reaction of the ingredients of the medium with the compounds. After 24 hr of incubation, the broth in each dilution tube was streaked onto full-strength trypticase soy agar in Petri plates and incubated for 24 hr to determine the presence or absence of bacterial colonies.

(15) F. Klivenyi, *Magy. Kem. Folyoirat*, **64**, 121 (1958); *Chem. Abstr.*, **54**, 16416 (1960).

(16) T. J. Markie and J. E. McCartney, "Handbook of Practical Bacteriology," 8th ed, Williams and Wilkins Co., Baltimore, Md., 1948, p 269.

The Effect of Some Disulfides and Thiols on the Carbohydrate Metabolism of Ehrlich Ascites Tumor[1]

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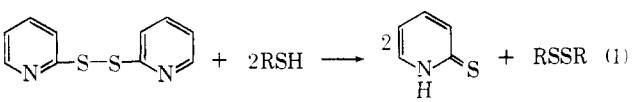
It has recently been found in this laboratory that 2,2′-dithiodipyridine inhibits the respiration and glycolysis of Ehrlich ascites tumor.[2] Further studies[3] have shown that 2,2′-dithiodipyridine and 4,4′-dithiodipyridine react readily and irreversibly with thiols,



as shown in eq 1 and that this reaction, when occurring in metabolically active cells (mouse liver and kidney, Ehrlich ascites tumor), can also cause the enzyme-mediated oxidation of nonsulfhydryl metabolites, such as glucose 6-phosphate.[4,5]

In the present communication we report further studies on the effect of a number of other disulfides, aliphatic and heterocyclic, on the carbohydrate metabolism of Ehrlich ascites tumor. In all cases, the effect of the corresponding thiol has also been studied.

Results and Discussion

Table I reports the results of the manometric experiments. Most of the thiols (or thiones) studied have no significant effect on Ehrlich ascites tumor metabolism; only cysteamine (I) and *N,N*′-dimethylcysteamine (II) cause a substantial inhibition of Q_{O_2}. In addition, I stimulates Q_{O_2}(G) and $Q^{O_2}_{CO_2}$ and inhibits $Q^{N_2}_{CO_2}$ if sufficient time is allowed.

Among the disulfides, it is apparent that two types of action can be distinguished: (a) strong inhibition of glycolysis and of respiration by XIII, XIV, XVII, XVIII, XXI, and XXII (the effect of these compounds is similar to that of 2,2′-dithiodipyridine);[2] and (b) moderate or negligible inhibition of anaerobic glycolysis and of respiration, accompanied by apparent stimulation of aerobic glycolysis (accumulation of lactate in the presence of oxygen) by XV, XVI, XIX, and XXIII.

(1) (a) This investigation was supported by Public Health Service Research Grant CA 08538-01, from the National Cancer Institute. (b) Part of the data reported here have been presented at the 3rd International Meeting of Medicinal Chemistry (Chimie Thérapeutique), Paris, France, July 1967.

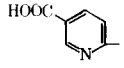
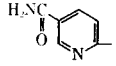
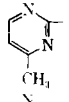
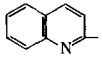
(2) D. R. Grassetti and J. F. Murray, Jr., *J. Med. Chem.*, **8**, 753 (1965).

(3) D. R. Grassetti and J. F. Murray, Jr., *Arch. Biochem. Biophys.*, **119** 41 (1967).

(4) D. R. Grassetti and J. F. Murray, Jr., *Anal. Biochem.*, in press.

(5) D. R. Grassetti and J. F. Murray, Jr., *Biochem. Pharmacol.*, in press.

TABLE I[a]

R	Thiols, RSH: Compd	Ref prepn	Concn, 10^3 M	% change: Q_{O_2}	$Q_{O_2}(G)$	$Q^{O_2}_{CO_2}$	$Q^{N_2}_{CO_2}$	Disulfides, RSSR: Compd	Ref prepn	Concn, M	Type effect	% change: Q_{O_2}	$Q_{O_2}(G)$	$Q^{O_2}_{CO_2}$	$Q^{N_2}_{CO_2}$
$H_2NCH_2CH_2$	I	*b*	5	1 hr, −27 2 hr, −97	+31 +35	−14 +48	−19 −52	XIII	*b*	5×10^{-3}	A	−90	−85	−80	−90
$(CH_3)_2NCH_2CH_2$	II	*b*	5	1 hr, −54 2 hr, −64	NSC[c] NSC	NSC NSC	NSC NSC	XIV	*g*	5×10^{-3}	A	−90	−80	−90	−90
$CH_3CONHCH_2CH_2$	III	*d*	5	+9	+15	+8	+2	XV	*d*	5×10^{-3}	B	−45	−5	+35	0
$HOOCCH_2CH_2$	IV	*b*	5	−6	+8	−7	+4	XVI	*b*	5×10^{-3}	B	−50	−60	+95	−20
	V	*b*	1	−19	0	0	−3	XVII	*h*	1×10^{-3}	A	−95	−90	−95	−95
	VI	*b*	1	−3	−5	−17	−9	XVIII	*i*	1×10^{-3}	A	−95	−90	−80	−90
	VII	*e*	5	+5	+4	+19	+7	XIX	New	5×10^{-3}	B	−80	−35	+23	−25
	VIII	*f*	1	−1	+10	+4	−7	XX	New	3.5×10^{-5}		−3	+10	+2	−9
	IX	*b*	1	−1	+1	−4	+1	XXI	New	1×10^{-3}	A	−95	−85	−90	−95
	X	*b*	1	+4	−5	+5	0	XXII	*j*	1×10^{-3}	A	−90	−85	−90	−95
	XI	*b*	1	−4	−3	+5	0	XXIII	*k*	1×10^{-3}	B	−50	−10	+35	−50
	XII	*b*	1	−5	+1	+39	−1	XXIV	*l*	3.2×10^{-6}		+3	−5	+1	+6

[a] Experiments were carried out at 37° for 1 hr, except when indicated otherwise. *Q* values are μl/mg dry weight of ascitic fluid per hr. Q_{O_2} = rate of O_2 uptake in air; $Q_{O_2}(G)$ = rate of O_2 uptake in air in the presence of 0.05 *M* glucose; $Q^{O_2}_{CO_2}$ = rate of CO_2 evolution in O_2-CO_2 (95:5) in the presence of 0.05 *M* glucose; $Q^{O_2}_{CO_2}$ = rate of CO_2 evolution in N_2-CO_2 (95:5) in the presence of 0.01 *M* glucose. [b] Commercial products. [c] NSC means no significant change was noted. These values were found to vary within a wide range (−30 to +47) in eight different experiments. [d] F. J. McQuillin and J. Stewart, *J. Chem. Soc.*, 2966 (1955). [e] C. Räth, *Ann.*, **487,** 105 (1931). [f] H. S. Forrest and J. Walker, *J. Chem. Soc.*, 1939 (1948). It was found advantageous to carry out the reaction in dimethylformamide instead of ethanol. [g] T. Suzuki, K. Imaeda, M. Kubota, and H. Takagi, *Japan. J. Pharm. Chem.*, **22,** 464 (1950); *Chem. Abstr.*, **45,** 8458*e* (1951). [h] A. M. Comrie and J. B. Stenlake, *J. Chem. Soc.*, 1853 (1958). [i] Olin Mathieson Chemical Corp., German Patent 1,224,744 (Sept. 15, 1966). [j] See Experimental Section. [k] W. H. Miller, R. O. Roblin, and E. B. Astwood, *J. Am. Chem. Soc.*, **67,** 2201 (1945). [l] T. Kametani, K. Fukumoto, and O. Umezawa, *Japan. J. Pharm. Chem.*, **31,** 132 (1959); *Chem. Abstr.*, **54,** 11019*a* (1960); see also S. Kubota and T. Akita, *J. Pharm. Soc. Japan*, **81,** 515 (1961); *Chem. Abstr.*, **55,** 19926*g* (1961).

TABLE II

SOLUBILITIES IN KREBS-RINGER PHOSPHATE BUFFER AT 37°

Compd	λ_{max}, mμ	$\epsilon \times 10^{-4}$	Soly, M
XII	216, 276, 384	5.0, 2.1, 1.3	1.4×10^{-3}
XX	252, 291	1.7, 1.7	3.5×10^{-5}
XXI	237	1.9	4.2×10^{-3}
XXII	240	1.9	2.4×10^{-3}
XXIV	210, 252, 320, 332	6.6, 5.1, 1.3, 1.6	3.2×10^{-3}

Within the group of compounds studied, type a effect is given by aliphatic or heterocyclic disulfides containing amino groups. If the basicity of the amino nitrogens is decreased, as for instance by N-acetylation of cystamine (to give XV), or by introduction of appropriate ring substituents in heterocyclic bases (to give XIX or XXIII), the compounds acquire type b effect. A similar change is obtained when the amino groups of cystamine are replaced by carboxyls (to give XVI). It is interesting to note that formation of the N-oxide (to give XVIII) does not alter the type a effect of 2,2′-dithiodipyridine.

The solubility of XX and XXIV was very low; a concentration comparable to that of the other disulfides studied could not be attained. At the maximum possible concentration these two compounds had no significant effect on the properties studied.

The accumulation of lactate from glucose, caused by action of type b compounds on Ehrlich ascites cells in air, indicates that the formation of pyruvate through the glycolytic pathway is not prevented by these compounds. However, the further oxidation of pyruvate through the Krebs cycle is undoubtedly inhibited. There appears thus to be a selective inhibition of the Krebs cycle by type b compounds, while the glycolytic pathway is relatively undisturbed. Compounds of type a, on the other hand, are strong inhibitors of the glycolytic pathway. The study of these compounds at the enzyme and molecular level is being continued.

Skrede, *et al.*,[6] have studied the effect of several disulfides on citrate oxidation by rat liver mitochondria. It is interesting to note that compounds which we classify as type a were inhibitory, whereas compounds of type b were not. Thus, cystamine at 2×10^{-3} M inhibited respiration to the extent of 70% in the first hour; on the other hand, at the same concentration, N,N′-diacetylcystamine caused no inhibition of mitochondrial oxidation of citrate.

Experimental Section

Materials and Methods. Manometry.—These experiments were carried out as reported previously,[2] except that, for the aerobic glycolysis, instead of lactate determination, the CO_2 evolution in a atmosphere of O_2-CO_2 (95:5) was determined in Krebs-Ringer bicarbonate buffer, pH 7.4. The amount of heparin added was 50 U.S.P. units/ml of ascitic fluid.

Solubility.—These experiments were carried out as described previously[2] and are reported in Table II.

Melting points were determined on the Fisher-Johns block.

6,6′-Dithiodinicotinic Acid (XIX).—6-Mercaptonicotinic acid was oxidized with iodine and KI at pH 7, according to the procedure described by Fox and Gibas.[7] The disulfide was purified by repeated extraction with hot acetone; mp 265°, quantitative yield.

Anal. Calcd for $C_{12}H_8N_2O_4S_2$: C, 46.75; H, 2.62. Found: C, 47.11; H, 2.82.

6,6′-Dithiodinicotinamide (XX).—6-Mercaptonicotinamide was oxidized with iodine and KI in alkaline medium (KOH), according to the procedure described by Miller, *et al.*[8] The disulfide was recrystallized from 2-propanol; mp 263–265°, yield 60%.

Anal. Calcd for $C_{12}H_{10}N_4O_2S_2$: C, 47.04; H, 3.29. Found: C, 46.34; H, 3.57.

2,2′-Dithiodipyrimidine (XXI).—2-Mercaptopyrimidine was oxidized with iodine and KI in alkaline medium.[8] The product was recrystallized from ethyl acetate–petroleum ether (bp 60–110°); mp 139–140°, yield 53%.

Anal. Calcd for $C_8H_6N_4S_2$: C, 43.22; H, 2.72. Found: C, 43.57; H, 2.95.

6,6′-Dimethyl-2,2′-dithiodipyrimidine (XXII).[9]—2-Mercapto-6-methylpyrimidine was oxidized in the same manner.[8] The product was recrystallized from acetone–petroleum ether (bp 30–60°); mp 108–109°, yield 90%.

Anal. Calcd for $C_{10}H_{10}N_4S_2$: C, 47.96; H, 4.02. Found: C, 48.36; H, 3.90.

Acknowledgments.—The authors wish to thank Dr. S. Abraham for helpful discussions and Miss H. T. Ruan for assistance in the manometric experiments.

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(7) H. H. Fox and J. T. Gibas, *J. Org. Chem.*, **23**, 64 (1958).

(8) E. Miller, F. S. Crossley, and M. L. Moore, *J. Am. Chem. Soc.*, **64**, 2322 (1942).

(9) Use of this compound for anticancer testing has been reported by H. M. Rauen and R. Nonhof, *Arzneimittel-Forsch.*, **13**, 558 (1963). No procedure or physical properties were given.

Amides of N-Acylcysteines as Mucolytic Agents

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The synthesis of N-acylated cysteines[1-3] as mucolytic agents was extended to include some new carboxamides,[4,5] typified by L-2-acetamido-3-mercaptopropionamide (**2**), the amide of N-acetyl-L-cysteine (NAC). Only two 2-amino-3-mercaptopropionamides have been reported[6,7] previously. 2-Amino-N-β-naphthyl-3-mercaptopropionamide[6] was prepared in connection with oxytocin studies, and 2-amino-3-mercapto-N-*n*-octadecylpropionamide[7] was obtained in crude form for use as an emulsifying agent.

Chemistry.—Despite unsuccessful attempts by earlier investigators[8,9] to obtain L-cystine diamide dihydrochloride (**19**)[9] by ammonolysis of L-cystine dimethyl ester dihydrochloride,[10] we have found that **19** can be isolated in good yield, provided complete conversion to the dihydrochloride is assured by acidification with

(1) T. A. Martin and C. W. Waller, U. S. Patent 3,184,505 (1965).

(2) T. A. Martin, J. R. Corrigan, and C. W. Waller, *J. Org. Chem.*, **30**, 2839 (1965).

(3) T. A. Martin, D. H. Causey, and J. R. Corrigan, manuscript in preparation.

(4) A detailed study of many of these compounds, and their precursors, as potential amino acid antagonists will be reported elsewhere.

(5) A. L. Sheffner, U. S. Patent 3,252,866 (1966), has disclosed the use of these compounds in hair waving compositions.

(6) H. Tuppy, U. Wiesbauer, and E. Wintersberger, *Monatsh.*, **94**, 321 (1963); A. Lindner, E. Stoklaska, and E. Wintersberger, *Chem. Abstr.*, **61**, 15940a (1964).

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(10) L. Zervas and I. Photaki, *J. Am. Chem. Soc.*, **84**, 3887 (1962).