Brit. J. Pharmacol. (1956), **11**, 351.

STUDIES IN THE CHEMOTHERAPY OF TUBERCULOSIS: ETHYL MERCAPTAN AND RELATED COMPOUNDS

BY

G. E. DAVIES, G. W. DRIVER, E. HOGGARTH, A. R. MARTIN, M. F. C. PAIGE, F. L. ROSE, AND B. R. WILSON

From the Imperial Chemical (Pharmaceuticals) Limited Research Department, Blackley, Manchester

(RECEIVED FEBRUARY 6, 1956)

Del Pianto (1950) claimed that a mixture of sodium 2-mercaptobenzthiazole-5-sulphonate and sodium *S*-ethylthiosulphate prevented the development of tuberculosis in infected guinea-pigs, and that neither compound alone showed antituberculous activity. The sodium 2-mercaptobenzthiazole-5-sulphonate could be replaced by other benzthiazoles and the sodium *S*-ethylthiosulphate by other alkylthiosulphates, but with the latter smaller "effects" were produced. Sodium *S*-ethylthiosulphate was tried because of its relation to cysteine, which had been found to potentiate the antibacterial activity of the benzthiazoles against *Staphylococcus aureus*.

While investigating this claim, we found that, though sodium 2-mercaptobenzthiazole-5-sulphonate was without antituberculous activity in infected mice or guinea-pigs, sodium *S*-ethylthiosulphate was very active.

The purpose of this communication is to report the development of this discovery. Since the completion of this work, the antituberculous activity of ethyl mercaptan compounds has been reported in the U.S.A. by two groups of workers (Brown, Matzuk, Becker, Conbene, Constantin, Solotorovsky, Winsten, Ironson, and Quastel, 1954; Kushner, Dalalian, Bach, Centola, Sanjurjo, and Williams, 1955), and, in addition, an abstract of a paper by Del Pianto (1953) has appeared in which he claims that, contrary to his earlier observations, sodium *S*-ethylthiosulphate is a highly potent antituberculous agent in experimental animals.

METHODS

Male albino mice weighing 18–22 g. were infected intravenously with 0.75 mg. wet weight of *Mycobacterium tuberculosis*, human type, strain 905, suspended in 0.1 ml. of distilled water. The suspension was made by ball-milling bacilli harvested from a 14-day culture on Löwenstein's medium. Compounds were administered orally twice daily, or subcutaneously once daily, to groups of 6 or 10 mice for two weeks. In each experiment a group of mice was treated with either streptomycin, 50 or 100 mg./kg., subcutaneously once daily, or isoniazid, 7.5 mg./kg., incorporated in powdered food.

With this type of infection untreated mice died with mean survival times ranging from about 18 to about 24 days. Streptomycin at 100 mg./kg. increased the mean survival time by about 9 days and isoniazid at 7.5 mg./kg. increased it by about 25 days.

RESULTS

Sodium *S*-ethylthiosulphate was the only active member of a series of sodium thiosulphates tested. The following *S*-substituted monosodiumthiosulphates were inactive: methyl-, *n*-propyl-, *iso*-propyl-, allyl-, *n*-amyl-, *n*-docedyl-, β-hydroxyethyl-, carbethoxymethyl-ethylene-bis-, trimethylene-bis-, benzyl-, phenyl-, *p*-chlorophenyl-, *p*-methoxyphenyl-, *p*-phenylenediamine-2-5-bis-. Sodium thiosulphate itself was also inactive.

Sodium *S*-ethylthiosulphate decomposed on storage to give diethyldisulphide which showed high antituberculous activity (Table I). The other disulphides shown in Table I were also effective,

TABLE I

DISULPHIDES ($R-S-S-C_2H_5$) ACTIVE AGAINST TUBERCULOSIS IN MICE

R	Dose (mg./20 g.)	Increased Mean Survival Time (Days)	Increase Required for Significance (Days)
C_2H_5—	20 p.o.	4·6	2·1
	2·5 s.c.	20·6	2·1
n-C_3H_7—	0·8 ,,	16·0	5·0
	0·4 ,,	5·0	5·0
Cl-C$_6$H$_4$—	0·6 ,,	13·0	5·0
	1·2 ,,	14·1	5·0
Cl_3C—	0·65 ,,	1·1	2·5
	1·3 ,,	9·4	2·7
—$CH_2CHNH_2.CO_2H$	5·0 p.o.	6·3	2·1
	10·0 ,,	13·7	2·4

whereas dimethyl-, di-*n*-propyl-, di-*iso*propyl-, didodecyl-, di-carboxymethyl-, di-*p*-nitrophenyl-, and di-3:4-diaminophenyl-disulphides were inactive.

Since sodium *S*-ethylthiosulphate and the active disulphides could be decomposed *in vitro*, and presumably *in vivo*, to give ethyl mercaptan, we tested this substance and found it to be active in mice (Table II). The following related thiols were inactive: *n*-propane-, *n*-butane-, *iso*butane-, *n*-heptane-, β-aminoethane-, β-diethylaminoethane-, β-(1-piperidyl) ethane-, β-(4-morpholino) ethane-, β-anilinoethane-, *N*:*N*-anilinobis-β:β′-ethane-, *di*-β-hydroxyethane-, β-carboxymethane-, butane-1:4-di-, ethane-1:2-di-, *cyclo*hexane-, benzene-, 4-diethylamino-2-aminobenzene-, 4-hydroxypyrimidine-2-, 4-methyl-2-aminopyrimidine-4-, 2-phenyl-4-hydroxy-1:3:5-triazine-6-, 2-amino-4-hydroxylamino-1:3:5-triazine-6-, 2-hydroxy-1:3:5-triazine-4:6-di-, 4-nitrobenzimidazole-2-thiols; DL-penicillamine, DL-penicillamine ethyl ester, 2:3-dimercaptopropanol (BAL).

TABLE II

EFFECT OF ETHYL MERCAPTAN ON TUBERCULOSIS IN MICE

Dose (mg./20 g.)	Increased Mean Survival Time (Days)	Increase Required for Significance (Days)
5 p.o. 4 times daily	6·0	2·1
10 s.c. daily for 15 days ..	32·8	6·7
60 in drinking water for 15 days	42·2	6·7

We had thus established that ethyl mercaptan, or a source of ethyl mercaptan, was antituberculous in mice and that this property was peculiar to the ethyl homologue. Yet, as is shown below, the mercaptan had only a slight action on tubercle bacilli *in vitro*. These observations, taken in conjunction with the known chemical reactivity of thiols, suggested that a metabolite of ethyl mercaptan was the ultimate active agent. Two possible mechanisms of the metabolism of ethyl mercaptan were envisaged: (*a*) oxidative, and (*b*) that the thiol may act as an ethylating or "ethylthiolating" agent.

Oxidative Metabolism.—This possibility includes compounds formed by oxidation at the sulphur atom (which may have undergone prior methylation) giving sulphoxides, sulphones, sulphinic and sulphonic acids; compounds formed by oxidation at the 2-carbon atom, giving derivatives of ethanol, acetaldehyde or acetic acid, and compounds formed by oxidation at both these centres. Accordingly the following substances were tested: ethyl

TABLE III

EFFECT OF ETHYL THIOL-ESTERS OF CARBOXYLIC ACIDS ON TUBERCULOSIS IN MICE

Acid	Dose (mg./20 g.)	Increased Mean Survival Time (Days)	Increase Required for Significance (Days)
Acetic	20 p.o.†	4·6	2·6
Propionic	0·35 s.c. 0·7 ,,	1·3 8·6	1·7 1·7
n-Butyric	0·35 ,, 0·7 ,,	3·3 8·2	1·7 1·7
n-Caproic	0·5 ,, 1·0 ,,	3·1 23·3	1·7 1·7
Lauric	0·75 ,, 1·5 ,,	2·7 1·1	1·7 1·7
Dimethylaminoacetic	5·0 p.o. 10·0 ,,	6·3 11·0	2·3 2·3
Malonic (bis ester)	5·0 ,, 10·0 ,,	7·0 13·4	1·8 4·6
,, (mono ester)	0·45 s.c. 0·9 ,,	0·1 0	2·3 2·4
Hippuric	5·0 p.o. 10·0 ,,	5·0 7·6	2·5 2·3
Phenylacetic	1·0 s.c. 5·0 ,, 5·0 p.o. 10·0 ,,	32·8 35·1 10·3 12·3	 2·0 2·0
Cinnamic	1·0 s.c. 2·0 ,,	−3·6 −1·3	5·0* 5·0
Benzoic	1·0 ,, 1·0 ,, 8·0 p.o.†	10·0 40·4 9·3	2·6 12·0* 3·1
Acetylsalicylic	1·0 s.c. 10·0 ,,	1·5 14·7	2·5 2·5
Phthalic	4·0 p.o.† 8·0 ,, †	2·2 8·1	3·1 3·2
m-Nitrobenzoic	8·0 ,, † 20·0 ,, †	7·2 37·4	3·1 3·1
m-Aminobenzoic	1·0 s.c.	16·0	12·0*
iso-Phthalic	10·0 p.o. 10·0 s.c. 5·0 ,,	46·7 >83·0 58·4	
p-Toluic	8·0 p.o. † 20·0 ,, †	13·1 25·7	3·2 3·1
p-Anisic	8·0 ,, † 20·0 ,, †	6·1 28·4	3·1 3·1
p-Nitrobenzoic	2·0 ,, 4·0 ,,	3·4 16·2	5·9 5·9
p-Aminobenzoic	1·0 s.c. 2·0 p.o.† 4·0 ,, †	0·7 1·7 2·0	1·8 2·1 2·1
p-Dimethylaminobenzoic ..	2·5 ,, 5·0 ,, 5·0 ,,	5·7 8·3 22·2	4·4 8·3 12·0*
p-Ethanesulphonylbenzoic ..	8·0 ,, † 20·0 ,, †	4·7 12·7	2·5 2·5
p-Aminosulphonylbenzoic ..	0·75 ,, 1·5 ,,	2·3 4·0	2·3 2·3
*Tere*phthalic	0·7 s.c. 1·5 ,, 20·0 p.o.†	7·8 10·5 10·4	2·6 2·8 2 0

TABLE III—*continued*

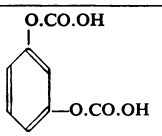
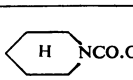
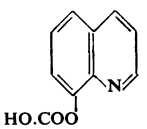
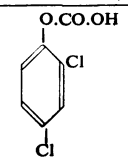
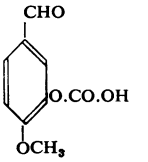
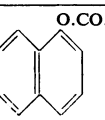
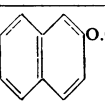
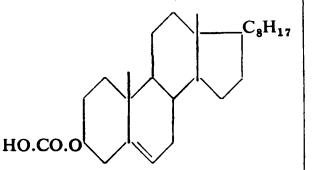
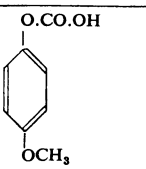
Acid	Dose (mg./20 g.)	Increased Mean Survival Time (Days)	Increase Required for Significance (Days)
*Tere*phthalic (mono ester) ..	20·0 p.o.† 8·0 ,,†	0 0	1·8 1·8
2: 4-Dichloro-benzoic	1·5 s.c.	7·3	2·6
iso-Nicotinic	1·0 ,, 10·0 ,, 8·0 p.o.† 20·0 ,,†	1·5 14·7 15·3 17·8	2·5 2·5 5·3 4·7
Carbonic	1·0 s.c. 2·0 ,,	10·6 10·6	1·7 1·7
$CH_3O.CO.OH$	0·35 ,, 0·7 ,,	2·9 2·8	2·3 2·3
$C_2H_5O.CO.OH$	2·0 p.o.† 4·0 ,,†	−0·7 5·8	2·7 2·7
n-$C_4H_9O.CO.OH$	0·5 s.c. 1·0 ,,	1·8 23·3	2·3 2·3
tert.-$C_4H_9O.CO.OH$	0·5 ,, 1·0 ,,	1·2 17·0	2·4 2·4
$C_2H_5O.CH_2CH_2O.COOH$ ..	0·55 ,, 1·1 ,,	2·1 6·4	2·3 2·3
$C_6H_{11}O.CO.OH$	0·55 ,, 1·1 ,,	1·5 10·8	2·3 2·3
$C_6H_5O.CO.OH$	0·55 ,, 1·1 ,,	6·4 23·4	2·3 2·3
	0·43 ,, 0·86 ,,	1·0 7·2	2·3 2·3
Carbamic	1·0 p.o. 2·0 ,,	2·7 4·6	1·7 1·7
Diethylcarbamic	1·0 ,, 2·0 ,,	−0·5 0·2	2·3 2·3
	0·5 s.c. 1·0 ,,	1·3 1·0	1·7 1·7
$O_2NC(CH_2O.CO.OH)_3$	1·5 p.o. 3·0 ,,	3·1 4·1	2·3 2·3
$C(CH_2O.CO.OH)_4$	5·0 ,, 10·0 ,,	1·7 4·4	1·9 1·8
	5·0 ,, 10·0 ,,	3·3 5·8	2·2 2·3
	0·7 s.c. 1·4 ,,	0·3 4·4	1·8 1·8

TABLE III—*continued*

Acid	Dose (mg./20 g.)	Increased Mean Survival Time (Days)	Increase Required for Significance (Days)
	12 p.o. 20 ,,	3·8 5·6	2·5 2·5
	0·7 s.c. 1·4 ,,	0·8 11·1	1·9 1·9
	5·0 p.o. 10·0 ,,	6·3 14·8	2·2 2·2
	5·0 ,, 10·0 ,,	−1·2 −1·4	1·9 1·9
	0·65 s.c. 1·3 ,,	6·4 12·0	2·0 2·0
	1·0 p.o. 2·0 ,,	3·1 8·4	2·1 2·1

* Treatment delayed until 14 days after infection.
† Daily dose administered in food.

methyl sulphide, sulphoxide and sulphone ; diethylsulphide, sulphoxide and sulphone ; ethanesulphinic acid ethyl ester, *n*-butyl ester, *N* : *N*-diethylamide, *N* : *N*-dibutylamide, and piperidide ; ethanesulphonic acid ; trithio-acetaldehyde (α and β forms) ; β-hydroxyethanethiol ; β-hydroxyethylmethylsulphide and sulphone ; β-hydroxydiethylsulphide and sulphone ; bis-β-hydroxyethylsulphide and sulphoxide ; thioglycollic acid and amide ; S-methylthioglycollic acid ; thioacetic acid ; S-ethylethanethiosulphinic acid, S-ethylethanethiosulphonic acid and butane-1 : 4-dithiol. They were all without therapeutic effect.

Ethylation.—The possibility that ethyl mercaptan may act as an ethylating agent *in vivo* was considered by examining the ethyl analogues of

choline, methionine and betaine. Other possible ethylating agents, such as diethylsulphide, diethylsulphate, ethylsulphuric acid and ethylxanthate were included, but none of these substances was antituberculous. In addition, thioethanolamine and compounds related to it (and hence to Coenzyme A), such as ethanolamine, bis-(β-aminoethyl) disulphide, bis-(β-aminoethyl) sulphide and sulphone, methyl-β-aminoethyl sulphide, and bis-(β-diethylaminoethyl)-sulphides; and the vinylsulphones, vinyl methyl sulphone, divinyl sulphone and reaction products of these with the amines glycine, ethylamine, cysteine and *p*-aminobenzoic acid, were all examined as potential antituberculous agents derived from ethyl mercaptan. These compounds, also, were inactive.

It was concluded from this preliminary work, that a therapeutically active compound in this series would have to be a source of ethyl mercaptan. Because of the unpleasant smell of ethyl mercaptan it was desirable that a therapeutically acceptable compound should release the thiol only after absorption. The class of compounds most likely to supply a drug acceptable both pharmacologically and pharmaceutically seemed to be the thiol-esters. The first of these to be prepared, ethyl thiolbenzoate, showed outstanding activity, a result which led to the examination of a large number of compounds of this type. It will be seen from Tables III and IV that the specificity of the ethyl group was demonstrated yet again. The thiol-esters were invariably more active when administered parenterally than when administered orally.

Other potential sources of ethyl mercaptan have been examined (Table V), but no compounds superior to the thiol-esters have been discovered.

Experiments in vitro

Ethyl mercaptan inhibited the growth of tubercle bacilli in protein-free liquid media at a concentration of 1:20,000. The addition of 0.5% bovine plasma albumin increased the inhibitory concentration to 1:5,000, and in the presence of 10% unheated horse serum there was no inhibition at 1:5,000. Ethyl thiolbenzoate and diethyl dithiol-*iso*phthalate, both of which are highly active in mice, were virtually inactive *in vitro*. Sera from animals receiving high doses of active thiol-esters were without bacteriostatic activity when diluted 1:10 in liquid media, as were small pieces of liver, lung, spleen, and kidney.

TABLE IV

THIOL-ESTERS AND RELATED COMPOUNDS INACTIVE AGAINST TUBERCULOSIS IN MICE

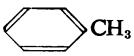
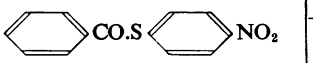
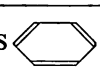
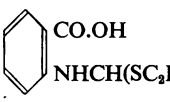
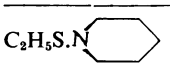
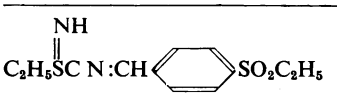
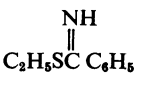
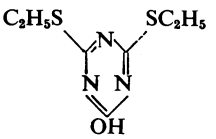
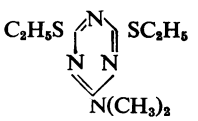
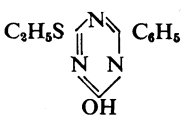
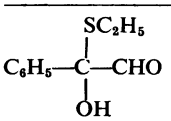
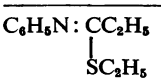
$CH_3CO.SCH_3$		$P(SC_2H_5)_3$
$C_6H_5CO.SCH_3$		$OP(SC_2H_5)_3$
		
$CH_3CO.SC_3H_7(n)$	$C_2H_5S.CS.SNa$	
$C_6H_5CO.SC_3H_7(n)$	$C_2H_5S.CS.SC_2H_5$	$C_6H_5SO_2.SC_6H_5$
$C_6H_5CO.SC_3H_7(iso)$	$C_2H_5O.CS.SC_2H_5$	$C_2H_5SO.SC_2H_5$
$CH_2S.CO.CH_3$	$(C_2H_5)_2NCS.SC_2H_5$	$C_3H_7SO.SC_3H_7(n)$
$CH_2S.CO.CH_3$	$NH_2NHCS.SC_2H_5$	$CH(SC_2H_5)_3$
$CH_2S.CO.C_6H_5$	$C_6H_5CH:NNHCS.SC_2H_5$	$C(SCH_3)_4$
$CH_2S.CO.C_6H_5$	$C_6H_{12}O_5:NNHCS.SC_2H_5$	$C(SC_2H_5)_4$
$CH_3CO.SC_6H_5$	$C_2H_5SO_2.SC_2H_5$	C_2H_5SCN
$C_6H_5CO.SC_6H_5$	$C_6H_5SO_2.SC_2H_5$	$C_2H_5N=C(SC_2H_5)_2$
		$C_6H_5CS.OC_2H_5$
		$C_6H_5CO.OC_2H_5$

TABLE V

EFFECT OF VARIOUS SOURCES OF ETHYL MERCAPTAN ON TUBERCULOSIS IN THE MOUSE

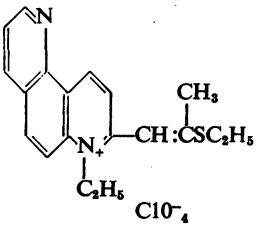
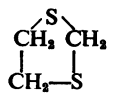
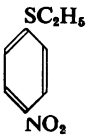
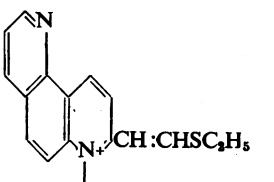
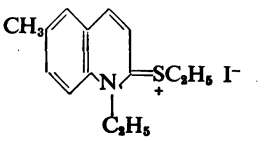
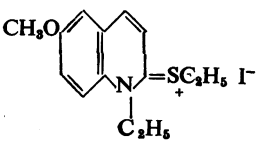
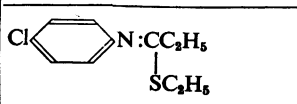
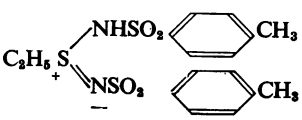
A. Active Compounds

	Dose (mg./20 g.)	Increased Mean Survival Time (Days)	Increase Required for Significance (Days)
$C_2H_5SCH_2CHNH_2.CO.OH$	4·0 p.o. 6·0 ,, 10·0 ,, 5·0 s.c.	3·2 0·1 7·8 −0·2	5.6 2·1 5·6
	5·0 p.o. 10·0 ,, 0·9 s.c.	3·8 5·8 2·2	1·8 1·8 2·0
$C_6H_5CH(SC_2H_5)CH(COCH_3)_2$	5·0 p.o. 10·0 ,,	1·4 2·6	1·8 1·9
	0·45 s.c. 0·9 ,,	1·6 6·1	2·6 2·6
	1·0 ,, 2·0 ,,	6·6 9·0	3·3 3·7
	8·0 p.o.* 20·0 ,, *	2·2 7·7	2·1 5·9
	2·5 ,, 5·0 ,,	1·9 3·9	2·7 2·7
	8·0 ,, * 20·0 ,, *	8·7 −0·8	6·7 6·7
	2·5 ,, 5·0 ,,	0·2 5·3	1·8 4·4
	2·0 ,, 5·0 ,,	2·1 7·4	1·8 1·8
	1·0 ,, 2·5 ,,	0 5·1	2·4 2·4

* Daily dose administered in food.

TABLE V—*continued*

B. Inactive Compounds

$CH_2(SC_2H_5)_2$	$CH_3COCH(CHSC_2H_5.C_6H_5)CO_2C_2H_5$	
$CH_3CH(SC_2H_5)_2$	$C_6H_5CHSC_2H_5.CH(CO_2C_2H_5)_2$	
$C_6H_{12}O_5$: $(SC_2H_5)_2$ D. Glucose diethyl acetal	$(C_6H_5CH(SC_2H_5)CH_3)_2CO$	
		
$(CH_3)_2C(SC_2H_5)_2$		
$CH_3CH_2OCH_2CH_2OCH_2SC_2H_5$		
$C_6H_5CH:CHCH(SC_2H_5).CH_2COC_6H_5$		
		

DISCUSSION

It is apparent from the foregoing results that ethyl mercaptan exerts a profound effect on the tuberculous process in infected mice. Any compound which can be expected to produce ethyl mercaptan either before or after absorption shows antituberculous activity, and this activity is specific for the ethyl radical, even closely related thiols being completely inactive. The antituberculous activity of ethyl mercaptan derivatives varies considerably, no doubt owing to variations in absorption and degradation, but the most attractive and consistently active group of derivatives consists of the ethyl thiol-esters. Further study has therefore been confined to these esters, and work on this topic will be reported separately.

SUMMARY

1. Ethyl mercaptan shows high antituberculous activity in the infected mouse.

2. No other thiols tested showed this activity.

3. Derivatives of ethyl mercaptan which can be metabolized to the parent substance are also active.

4. As potential drugs for the treatment of the human disease, the ethyl thiol-esters appear to be the most promising.

REFERENCES

Brown, H. D., Matzuk, A. R., Becker, H. J., Conbene, J. P., Constantin, J. M., Solotorovsky, M., Winsten, S., Ironson, E., and Quastel, J. H. (1954). *J. Amer. chem. Soc.*, **76**, 3860.

Del Pianto, E. (1950). *Ric. Sci.*, **20**, 83.

—— (1953). Ibid., **23**, 1785. Cited in *Chem. Abstr.*, 1954, **48**, 13989.

Kushner, S., Dalalian, H., Bach, F. L., Centola, D., Sanjurjo, J. L., and Williams, J. H. (1955). *J. Amer. chem. Soc.*, **77**, 1152.