THE ANTITUBERCULAR PROPERTIES OF A SERIES OF THIOLS AND SULPHIDES

BY

P. ACRED AND D. M. BROWN

From the Beecham Research Laboratories Brockham Park, Betchworth, Surrey

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The antitubercular activity of a series of thiols, dithiolans, thiol esters, dimercaptopropyl esters, and episulphides has been examined *in vitro* and *in vivo* in mice infected with the H₃₇Rv strain of *Mycobacterium tuberculosis*. Most of the thiol compounds were inactive, although dimercaprol (2,3-dimercaptopropanol; B.A.L.) and a few closely related compounds showed slight activity *in vivo*, the only exception being 2,3-dimercaptopropyl chloride which was very active. The dithiolans were inactive, but some of the thiol esters were moderately active, in particular 2,3-di(acetylthio)propyl acetate and 1,2,3-tri(acetylthio)propane. The majority of the dimercaptopropyl esters had significant activity, the most active compounds being 2,3-dimercaptopropyl benzoate, 1,3-dimercapto-2-propyl benzoate, 2,3-dimercaptopropyl *o*-chlorobenzoate, and 2,3-dimercaptopropyl *p*-chlorobenzoate. All the *S*-acyl derivatives of 3-mercaptopropylene sulphide had good antitubercular activity, some being more active than streptomycin. The most active compound of the series was 3-(2-furoylthio)propylene sulphide. The activity of the compounds is believed to be due to their conversion *in vivo* to 3-mercaptopropylene sulphide and not due to the formation of ethanethiol. Slight deviation from the basic structure abolishes antitubercular activity.

Anderson and Chin (1947) first drew attention to the role of thiol compounds in experimental tuberculosis. These workers found that 2,3-dimercaptopropanol (dimercaprol; B.A.L.) 100 µg./ml. inhibited the growth of *Mycobacterium tuberculosis* grown in Dubos medium. Del Pianto (1950) reported the activity of 2-mercaptobenzo-thiazole in combination with salts of the *S*-esters of thiosulphonic acid. He suggested that the antitubercular activity of sodium ethyl thiosulphate was due to the formation of ethylmercaptan (ethanethiol). Solotorovsky and his colleagues [Brown, Matzuk, Becker, Conbere, Constatin, Solotorovsky, Winsten, Ironson and Quastel (1954); Solotorovsky, Winsten, Ironson, Brown and Becker (1954); and Solotorovsky, Winsten, Ironson and Brown (1956)] examined a series of ethylthio compounds, in particular *S*-ethyl-L-cysteine, which was reported to be more active than *p*-aminosalicylic acid when administered in the diet. Other workers were unable to confirm these findings and further investigation revealed that the activity of *S*-ethyl-L-cysteine and other members of the series depended upon the formation of the volatile compound ethanethiol [Solotorovsky (1955) (1956); Solotorovsky, Ironson, Winsten (1956); Oginsky, Solotorovsky and Brown (1955) (1956)]. Kushner, Dalalian, Bach,

Centola, Sanjurjo and Williams (1955) and Davies, Driver, Hoggarth, Martin, Paige, Rose and Wilson (1956) have investigated more recently the antitubercular activity of series of compounds related to ethanethiol. The latter authors considered that only compounds which were converted to ethanethiol were active.

We have investigated the antitubercular activity of dimercaprol and a number of related compounds and have found some active derivatives. The activity of the compounds does not appear to depend upon the formation of ethanethiol.

METHODS

The *organism* employed was the human strain of *Mycobacterium tuberculosis*, H₃₇Rv, reference National Collection of Type Cultures 7416.

Compounds to be tested for *in vitro* activity were treated according to their solubility. Aqueous solutions were used when possible, but compounds which were only sparingly soluble in water were dissolved in 1 ml. ethoxyethanol and diluted 1:20 with distilled water. This ether possesses some growth-inhibitory activity against *Mycobacterium tuberculosis* at a concentration of 1:20, but the amount of ethoxyethanol in the final test concentration was never allowed to exceed 1:200.

Serial concentrations of the dissolved compounds were made in Dubos medium. The tubes were inoculated with two drops of 1:10 concentration of a

suspension, standardized to Wellcome opacity tube 2, of a 10-day-old culture of *Mycobacterium tuberculosis* grown in Dubos, and incubated at 37°. Readings of growth were made at 7 days.

The activity is recorded as the lowest concentration of the compound ($\mu\text{g./ml.}$) inhibiting growth of the organism, that is, the minimal inhibitory concentration (M.I.C.).

In vivo Tests.—Male albino mice weighing 18 to 22 g. were infected by injecting intravenously 0.2 ml./20 g. of a suspension in saline of a 15-day-old culture

of H₃₇Rv strain of *Mycobacterium tuberculosis* grown in Proskauer and Beck medium. The suspension opacity was adjusted to a density equivalent to 4 mg. wet weight of organism/ml.

Compounds were administered daily in arachis oil (unless otherwise stated—see Tables) at doses of 1/2, 1/5, 1/10, and 1/20th of their approximate subcutaneous LD₅₀ values to groups of mice, 10 to a group. The arachis oil solution acted as a depot from which the compounds were slowly absorbed. In each experiment one group received no treatment and acted

TABLE I
INACTIVE THIOLS

B.R.L. No.	Formula	B.R.L. No.	Formula
215	$\text{CH}_2(\text{SH}).\text{CH}_2.\text{SH}$	554	$\begin{array}{c} \text{S.CH}_2.\text{CH}(\text{SH}).\text{CH}_3 \\ \\ [\text{CH}_2]_3 \\ \\ \text{S.CH}_2.\text{CH}(\text{SH}).\text{CH}_3 \end{array}$
217	$\text{CH}_2(\text{SH}).\text{CH}_2.\text{CH}_2.\text{SH}$		
218	$\text{CH}_2(\text{SH}).\text{CH}_2.\text{CH}_2.\text{CH}_2.\text{SH}$	559	$\text{CH}_3.\text{CH}(\text{SH}).\text{CH}_2.\text{S}.[\text{CH}_2]_3.\text{SH}$
231	$\text{CH}_2(\text{SH}).\text{CH}(\text{OH}).\text{CH}_2.\text{SH}$	560	$\text{CH}_2(\text{OH}).\text{CH}_2.\text{CH}_2.\text{S.CH}_2.\text{CH}(\text{SH}).\text{CH}_3$
243	$\text{CH}_2(\text{OH}).\text{CH}_2.\text{SH}$	570	$\text{CH}_2(\text{SH}).\text{CH}_2(\text{OH}).\text{CH}_2.\text{S.CH}_2.\text{CH}(\text{SH}).\text{CH}_3$
244	$\text{CH}_2(\text{SH}).\text{CH}(\text{SH}).\text{CH}_3$	573	$\text{CH}_2(\text{SH}).\text{CH}_2.\text{S.CH}_2.\text{CH}_2.\text{SH}$
248	$\text{CH}_2(\text{SH}).\text{CH}(\text{SH}).\text{CH}_2.\text{SH}$	576	$\text{CH}_2(\text{SH}).\text{CH}(\text{OH}).\text{CH}_2.\text{CH}_2.\text{SH}$
259	$\text{CH}_2(\text{OH}).\text{CH}_2.\text{CH}_2.\text{SH}$	581	$\text{CH}_3.\text{CH}(\text{SH}).\text{CH}(\text{SH}).\text{CH}_2.\text{OH}$
264	$\text{CH}_2(\text{SH}).\text{CH}(\text{OH}).\text{CH}_3$	584	$\text{CH}_2(\text{SH}).\text{CH}(\text{SH}).\text{CH}_2.\text{S.CH}_2.\text{CH}(\text{SH}).\text{CH}_3$
271	$\text{CH}_2(\text{OH}).\text{CH}(\text{OH}).\text{CH}_2.\text{SH}$	614	$\text{CH}_2(\text{SH}).\text{CH}(\text{O.CO.CH}_3).\text{CH}_2.\text{SH}$
449	$\text{CH}_2(\text{SH}).\text{CH}(\text{SH}).\text{CH}_2.\text{OC}_2\text{H}_5$	622	$\text{CH}_2(\text{SH}).\text{CH}_2.\text{CH}(\text{SH}).\text{CH}_2.\text{O.CO.CH}_3$
458	$\text{CH}_2(\text{SH}).\text{CH}(\text{SH}).\text{CO.OCH}_3$	633	$\text{C}(\text{CH}_2.\text{SH})_2(\text{OH})(\text{CO}_2\text{H})$
476	$\text{CH}_2(\text{SH}).\text{CH}(\text{SH}).\text{CH}(\text{OCH}_3).\text{CH}_2.\text{OCH}_3$	639	$\text{CH}_2(\text{OH}).\text{CH}(\text{SH}).\text{CH}(\text{SH}).\text{CH}_2.\text{OH}$
484	$\text{CH}_2(\text{SH}).\text{CH}(\text{SH}).\text{CO}_2\text{H}$	713	$\text{CH}_2(\text{SH}).\text{CH}(\text{SH}).\text{CH}_2.\text{O.C}_6\text{H}_5$
493	$\text{CH}_2(\text{SH}).\text{CH}(\text{OH}).\text{CH}(\text{SH}).\text{CH}_3$	735	$\text{CH}_2[\text{SH}].\text{CH}[\text{SH}].\text{CH}_2.\text{O} \cdot \text{C}_6\text{H}_4 \cdot \text{Cl}$
496	$\text{CH}_2(\text{SH}).\text{C}(\text{CH}_3)(\text{OH}).\text{CH}_2.\text{SH}$	756	$\text{CH}_2[\text{SH}].\text{CH}[\text{SH}].\text{CH}_2.\text{S} \cdot \text{C}_6\text{H}_4 \cdot \text{CH}_3$
515	$\text{CH}_2(\text{SH}).\text{CH}_2.\text{CH}(\text{SH}).\text{CH}_2.\text{OH}$	765	$\text{CH}_2(\text{SH}).\text{CH}(\text{OH}).\text{CH}(\text{SH}).\text{CH}_2.\text{SH}$
522	$\text{CH}_2(\text{SH}).\text{CH}(\text{SH}).\text{CH}(\text{OH}).\text{CH}_3$		
552	$\text{CH}_3.\text{CH}(\text{SH}).\text{CH}_2.\text{S.CH}_2.\text{CH}_2.\text{OH}$		

TABLE II
ACTIVE THIOLS

All compounds were administered subcutaneously in arachis oil.

B.R.L. No.	Formula	In vitro Activity μg./ml.	LD50 mg./kg.	Extension in TD50 over Control. Dose Fraction of LD50			
				1/2	1/5	1/10	1/20
—	Dimercaprol CH ₂ (SH).CH(SH).CH ₂ OH	50·0	100	9·5	7·0	—	—
479	CH ₂ (SH).CH(SH).CH ₂ Cl	6·25	100	—	24·0	16·0	9·0
489	$ \begin{array}{c} \text{CH}_2(\text{SH}).\text{CH}(\text{SH}).\text{CH}_2 \\ \qquad \qquad \qquad \searrow \\ \qquad \qquad \qquad \text{S} \\ \qquad \qquad \qquad \nearrow \\ \text{CH}_2(\text{OH}).\text{CH}(\text{SH}).\text{CH}_2 \end{array} $	25–50	> 500	—	14·0	8·0	3·5
550	CH ₃ .CH(SH).CH ₂ .S.CH ₂ .CH ₂ SH	> 100·0	200	10·0	7·0	2·5	—
555	$ \begin{array}{c} \text{CH}_2(\text{OH}).\text{CH}(\text{SH}).\text{CH}_2 \\ \qquad \qquad \qquad \\ \text{CH}_3.\text{CH}(\text{SH}).\text{CH}_2.\text{S} \end{array} $	—	250	7·5	4·0	2·0	—
567	CH ₂ (SH).CH ₂ (SH).CH(Cl).CH ₃	50·0	500	11·0	7·0	3·5	—
578	CH ₂ (SH).C(CH ₃)(Cl).CH ₂ SH	> 50·0	400	—	8·0	6·0	—
609	CH ₃ .CH(SH).CH(SH).CH ₂ Cl	> 50·0	300	—	4·0	6·0	—
784	CH ₂ (SH).[CH ₂] ₈ .CH(SH).CH ₂ OH	> 50·0	500	—	4·0	0·5	—

as an infected control. Some compounds were tested for oral activity, either by administration by stomach tube or in the diet. The doses administered were again 1/2, 1/5, 1/10, and 1/20th of their approximate acute oral LD50. The water-soluble compounds were given in aqueous solution in a volume of 0.2 ml./20 g. and the insoluble solids as acacia suspension, 0.2 ml./20 g. When the compounds were given in the diet the solution or suspension was added to 250 g., so that the appropriate dose was contained in 5 g., that is, the average daily intake for one mouse. Daily dosing commenced in all cases 24 hr. after infection and continued until the twentieth day of the experiment. The TD50 values (median survival time) were determined by plotting the accumulative percentage mortality against time in days on log probit paper, and in cases where there was an increase in TD50 value over that of the control the results were analysed by the method of Litchfield (1949).

Toxicities.—Only approximate estimates of the acute LD50 values were obtained. The compounds were prepared as oil and water emulsions using acacia as the dispersing agent, and injected subcutaneously into groups of 5 mice per dose level.

RESULTS

Thiols

The inactive thiols are shown in Table I and the active thiols in Table II. The parent compound of the series, dimercaprol (2,3-dimercapto-propanol), and all the active thiols, apart from 2,3-dimercaptopropyl chloride (B.R.L. 479), were only slightly active *in vivo*. *In vitro* their activity was negligible. The compounds which were active possessed free thiol groups and a hydroxyl group, but not necessarily all compounds of this type were active. Acylation of the hydroxyl group or replacement of the hydroxyl group with a thiol, carboxyl or substituted carboxyl group abolished activity. Compounds having only one thiol group were inactive. If the hydroxyl group in the compounds having two vicinal thiol groups was replaced by a chlorine atom, activity was retained and might in fact be enhanced, for example, 2,3-dimercaptopropyl chloride (B.R.L. 479) was appreciably active *in vivo*, giving a considerable extension of the survival time. *In vitro* it had an

activity of 6.25 $\mu\text{g./ml.}$, being the only member of this group which had a significant *in vitro* activity.

The chloro compounds B.R.L. 479, B.R.L. 567 and B.R.L. 609, which were prepared from the hydroxyl analogues and hydrochloric acid, may be contaminated with isomeric structures (Doyle, Holland, Mansford, Nayler, and Queen, 1960).

Dithiolans

Ring closure to form dithiolans resulted in complete loss of activity—Table III.

Thiol Esters

The inactive thiol esters are listed in Table IV and the active thiol esters in Table V. Some of the active compounds showed considerable *in vitro* activity. 2,3-Di(acetylthio)propyl acetate (B.R.L. 403) and 2,3-di(acetylthio)propanol (B.R.L. 464) are active at a concentration of 0.8 $\mu\text{g./ml.}$ 1,2,3-Tri(acetylthio)propane (B.R.L. 411) was also very active *in vitro*. These three compounds also showed good activity *in vivo*. Increasing the size of the acyl group to a butyryl group, however, led to a considerable decrease both of *in vivo* and *in vitro* activity. The substitution of other radicals also led to a diminished activity.

TABLE III
DITHIOLANS

B.R.L. No.	Formula	B.R.L. No.	Formula
401		613	Glucose derivative of dimercaprol
412		647
414		650

TABLE IV
INACTIVE THIOL ESTERS

B.R.L. No.	Formula
450	$\text{CH}_2(\text{S.CO.CH}_3)_3.\text{CH}(\text{O.CO.CH}_3).\text{CH}_2.\text{S.CO.CH}_3$
471	$\text{CH}_2(\text{O.CH}_2\text{COOH})\text{CH}(\text{S.CO.CH}_3).\text{CH}_2\text{S.CO.CH}_3$
543	$\text{CH}_2(\text{O.CO.CH}_3).\text{CH}(\text{S.CO.CH}_3).\text{CH}_2.\text{CH}_2.\text{S.CO.CH}_3$
545	$\text{CH}_3\text{CH}(\text{O.CO.CH}_3).\text{CH}(\text{S.CO.CH}_3).\text{CH}_2.\text{S.CO.CH}_3$
568	$\text{CH}_2(\text{S.CO.CH}_3)_3.\text{C}(\text{CH}_3)(\text{O.CO.CH}_3).\text{CH}_2.\text{S.CO.CH}_3$
583	$\text{CH}_2(\text{S.CO.CH}_3).\text{CH}(\text{O.CO.CH}_3).\text{CH}_2.\text{CH}_2.\text{S.CO.CH}_3$
585	$\text{CH}_2(\text{O.CO.CH}_3).\text{CH}(\text{S.CO.CH}_3).\text{CH}(\text{S.CO.CH}_3).\text{CH}_3$
640	$\text{CH}_2(\text{O.CO.CH}_3).\text{CH}(\text{S.CO.CH}_3).\text{CH}(\text{S.CO.CH}_3).\text{CH}_2.\text{O.CO.CH}_3$
657	$\text{C}(\text{CH}_2.\text{S.CO.CH}_3)_2(\text{O.CO.CH}_3)(\text{CO}_2\text{H})$
755	$\text{CH}_2(\text{S.CO.CH}_3).\text{CH}(\text{O.CO.CH}_3).[\text{CH}_2]_4.\text{S.CO.CH}_3$
766	$\text{CH}_2(\text{S.CO.CH}_3).\text{CH}(\text{S.CO.CH}_3).\text{CH}(\text{O.CO.CH}_3).\text{CH}_2.\text{S.CO.CH}_3$
788	$\text{CH}_2(\text{S.CO.CH}_3).[\text{CH}_2]_8.\text{CH}(\text{S.CO.CH}_3).\text{CH}_2\text{O.CO.CH}_3$
826	$\text{CH}_2(\text{S.CH}_3).\text{CH}_2.\text{S.CO.CH}_3$

TABLE V
ACTIVE THIOL ESTERS

Compounds were administered subcutaneously in arachis oil except where indicated as follows: (a) orally in aqueous suspension.

B.R.L. No.	Formula	In vitro Activity $\mu\text{g./ml.}$	LD50 mg./kg.	Extension in TD50 over Control. Dose Fraction of LD50		
				1/2	1/5	1/10
403	$\begin{array}{ccccc} \text{CH}_2 & - & \text{CH} & - & \text{CH}_2 \\ & & & & \\ \text{S} & & \text{S} & & \text{O} \\ & & & & \\ \text{CO.CH}_3 & & \text{CO.CH}_3 & & \text{CO.CH}_3 \end{array}$	0.8	200	15.25	9.0	2.5
411	$\begin{array}{ccccc} \text{CH}_2 & - & \text{CH} & - & \text{CH}_2 \\ & & & & \\ \text{S} & & \text{S} & & \text{S} \\ & & & & \\ \text{CO.CH}_3 & & \text{CO.CH}_3 & & \text{CO.CH}_3 \end{array}$	1.6	99	17.5	9.5	2.5
464	$\begin{array}{ccccc} \text{CH}_2 & - & \text{CH} & - & \text{CH}_2 \\ & & & & \\ \text{S} & & \text{S} & & \text{OH} \\ & & & & \\ \text{CO.CH}_3 & & \text{CO.CH}_3 & & \end{array}$	0.8	150	11.5	4.5	2.75
535	$\begin{array}{ccccc} \text{CH}_2 & - & \text{CH} & - & \text{CH}_2 \\ & & & & \\ \text{O.CO.C}_2\text{H}_5 & & \text{S.CO.C}_2\text{H}_5 & & \text{S.CO.C}_2\text{H}_5 \end{array}$	—	>500	15.0	2.0	2.0
536	$\begin{array}{ccccc} \text{CH}_2 & - & \text{CH} & - & \text{CH}_2 \\ & & & & \\ \text{O.CO.C}_3\text{H}_7 & & \text{S.CO.C}_3\text{H}_7 & & \text{S.CO.C}_3\text{H}_7 \end{array}$	6.25	500	2.0	-0.5	-0.5
562	$\begin{array}{ccccccc} \text{CH}_3 & \text{CH} & \text{CH}_2 & \text{S} & \text{CH}_2 & \text{CH}_2 & - & \text{CH}_2 \\ & & & & & & & \\ & \text{S.CO.CH}_3 & & \text{S.CO.CH}_3 & & \text{O.CO.CH}_3 & & \end{array}$	6.25	500	2.5	-1.0	-1.5
655	$\begin{array}{ccccc} \text{CH}_2 & - & \text{CH} & - & \text{CH}_2 \\ & & & & \\ \text{S.CO.CH}_3 & & \text{O.SO}_2 & & \text{CH}_3 \\ & & & & \\ & & \text{C}_6\text{H}_4 & & \text{CH}_3 \end{array}$	6.25-12.5	>500	—	14.0	11.5
			150 (a)	—	2.0	3.0 (a)
758	$\begin{array}{ccccc} \text{CH}_2 & - & \text{CH} & - & [\text{CH}_2]_3 - & \text{CH}_2 \\ & & & & & \\ \text{S.CO.CH}_3 & & \text{O.CO.CH}_3 & & \text{S.CO.CH}_3 & \end{array}$	>50.0	>500	—	4.0	1.5
877	$\begin{array}{ccccc} \text{CH}_2 & - & \text{CH} & - & \text{CH}_2 \\ & & & & \\ \text{S.CO.CH}_3 & & \text{S.CO.CH}_3 & & \text{O.CO.C}_6\text{H}_5 \end{array}$	—	>500	—	4.0	2.5

TABLE VI
INACTIVE DIMERCAPTOPROPYL ESTERS

B.R.L. No.	Formula
646	$\text{CH}_2(\text{SH}).\text{CH}(\text{SH}).\text{CH}_2.\text{O.CO.}[\text{CH}_2]_{14}.\text{CH}_3$
694	$\text{CH}_2[\text{SH}].\text{CH}[\text{SH}].\text{CH}_2.\text{O.CO.}$ $\text{N}[\text{CH}_3]_2$

TABLE VII
ACTIVE DIMERCAPTOPROPYL ESTERS

Compounds were administered subcutaneously in arachis oil except where indicated as follows: (a) orally in aqueous suspension, (b) orally in arachis oil.

General formula:

B.R.L. No.	R.X	In vitro Activity $\mu\text{g./ml.}$	LD50 mg./kg.	Extension in TD50 over Control. Dose Fraction of LD50			
				1/2	1/5	1/10	1/20
463	R-CO.CH ₃	3.125-6.25	78	15.0	8.0	4.4	—
504	R-CO.C ₂ H ₅	12.5	150	13.0	4.0	—	—
510	R-CO.C ₃ H ₇	25.0	400	13.0	8.5	1.5	—
587	R-CO.C ₆ H ₅	12.5	>500	28.4	18.6	16.4	9.0
			600 (a)	5.2	2.5	1.25 (a)	—
			—	-3.5	11.5	0 (b)	—
636		25.0	>500	20.0	29.0	22.8	10.7
			500 (a)	6.5	2.0	2.5 (a)	—
637		12.5	500	54.0	29.0	16.0	11.25
638	R-CO.CH ₂ .C ₆ H ₅	25.0	500	—	14.5	8.5	1.0
			>500 (a)	—	3.0	3.5 (a)	—
651		25.0	200	22.5	11.5	5.5	—
668		50.0	>500	—	9.5	2.5	—
670		>50.0	>500	—	8.0	0.5	—
671	R-CO.CH ₂ .S.C ₂ H ₅	25.0	300	—	8.0	3.5	—
673	R-CO.CH ₂ .S.CH ₂ .C ₆ H ₅	50.0	>500	14.0	10.5	5.0	--
			>500 (a)	3.0	1.0	-0.5 (a)	--
680		1.56	500	34.0	19.0	10.25	3.5
693	R-CO.[CH ₂] ₂ .S.CH ₃	25.0	500	—	8.5	6.0	—
707		25-50	150	—	3.0	0.75	--

Dimercaptopropyl Esters

The inactive compounds are given in Table VI and the active compounds in Table VII. When the hydroxyl group is esterified there is usually a considerable increase in antitubercular activity, particularly in compounds with vicinal thiol groups, and where the hydroxyl group is adjacent to these. 2,3-Dimercaptopropyl benzoate (B.R.L. 587), 2,3-dimercaptopropyl *o*-chlorobenzoate (B.R.L. 636) and 2,3-dimercaptopropyl *p*-chlorobenzoate (B.R.L. 637) are the most active of this particular type. The precise nature of the ester radical, however, does not seem to be of great importance in determining the degree of activity. All of the *O*-esters of dimercaprol were, however, comparatively inactive *in vitro*.

A notable exception to the general pattern is 2-mercapto-1-mercaptopmethylethyl benzoate (B.R.L. 680), which has the two thiol groups separated by the ester group. This compound is active both *in vitro* and *in vivo*. The isomer 2,3-dimercaptopropyl benzoate (B.R.L. 587) is relatively inactive *in vitro*.

Episulphides

By eliminating the elements of water from thiol compounds containing a vicinal hydroxyl group

TABLE VIII
INACTIVE EPISULPHIDES

General formula:

B.R.L. No.	R.X
509	R.CH ₃
511	R.CH ₂ Cl
512	R.CH ₂ .O.C ₂ H ₅
513	R.CH ₂ .S.C ₂ H ₅
514	R.CH ₂ .N(C ₂ H ₅) ₂
520	R.CH ₂ .S.CO.NH.C ₆ H ₅
723	R.CH ₂ .S.CH ₃

TABLE IX
ACTIVE EPISULPHIDES

Compounds were administered subcutaneously in arachis oil except where indicated as follows: (a) orally in aqueous solution, (b) orally in arachis oil, (c) in the diet.

B.R.L. No.	Formula (R=CH ₂ -CH-) S	<i>In vitro</i> Activity μg./ml.	LD50 mg./kg.	Extension in TD50 over Control. Dose Fraction of LD50			
				1/2	1/5	1/10	1/20
482	R.CH ₂ SH	12.5	44 88 (a)	—1.5	—3.0	—3.0 (c)	—
				17.2	17.0	14.5	4.0
				3.0	1.25	1.0 (a)	—
				5.5	—1.0 (b)	—	—
528	CH ₃ .CH-CH.CH ₂ SH S	100	200	—	2.5	1.5	—
553	R.CH ₂ .S.S.CH ₂ .R	—	500	3.5	18.0	14.0	—
577	CH ₂ -CH.CH ₂ .CH ₂ SH S	50.0	400	—	4.5	6.75	—
599	R.CH(SH).CH ₃	12.5–25.0	100	2.5	1.5	—1.0	—

TABLE X
EPISULPHIDES WITH S-ACYL GROUPS

Compounds were administered subcutaneously in arachis oil except where indicated as follows: (a) orally in aqueous suspension, (b) orally in arachis oil, (c) in the diet, (d) orally in acacia suspension, (e) subcutaneously in acacia suspension.

General formula:

B.R.L. No.	R.X	In vitro Activity $\mu\text{g./ml.}$	LD50 mg./kg.	Extension in TD50 over Control. Dose Fraction of LD50			
				1/2	1/5	1/10	1/20
459	R.CO.CH ₃	0.4-0.8	45	17.25	14.0	12.0	4.5
			110 (a)	9.25	6.25	0.5 (a)	—
				3.5	2.0 (b)	—	—
				-2.5	-1.5	-2.5 (c)	—
540	R.CO.C ₂ H ₅	1.56	100	21.0	17.5	11.0	—
546	$\begin{array}{c} \text{CH}_2 - \text{CH} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{S} \cdot \text{CO} \cdot \text{CH}_3 \\ \diagdown \quad \diagup \\ \text{S} \end{array}$	—	300	14.5	5.25	0.5	—
561	R.CO.C ₃ H ₇	6.25-12.5	100	14.5	13.5	7.0	—
571	$\begin{array}{c} \text{CH}_2 - \text{C}(\text{CH}_3) \cdot \text{CH}_2 \cdot \text{S} \cdot \text{CO} \cdot \text{CH}_3 \\ \diagdown \quad \diagup \\ \text{S} \end{array}$	50.0	300	2.5	2.7	1.5	—
580	R.CO.CH ₂ .CH ₂ CO ₂ H	25.0	100	— 7.5	11.5 3.5	5.0 4.0	—
			200 (a)	—	1.5	1.5 (a)	—
590	R.CO.CH ₂ Cl	25.0	60	16.0	17.5	16.3	6.0
			150 (a)	3.5	2.5	2.5 (a)	—
595	$\begin{array}{c} \text{CH}_2 - \text{CH} \cdot \text{CH}(\text{S} \cdot \text{CO} \cdot \text{CH}_3) \cdot \text{CH}_3 \\ \diagdown \quad \diagup \\ \text{S} \end{array}$	25-50	300	5.5	3.5	1.5	—
600	R.CO.[CH ₂] ₂ .CO.OCH ₃	1.56-3.125	100	17.5	6.5	12.0	—
601	$\begin{array}{c} \text{CH}_2 - \text{CH} \cdot [\text{CH}_2]_3 \cdot \text{S} \cdot \text{CO} \cdot \text{CH}_3 \\ \diagdown \quad \diagup \\ \text{S} \end{array}$	3.125	>500	17.5	10.0	1.5	—
602	R.CO.O.C ₂ H ₅	1.56	150	16.5	18.0	15.0	4.5
605	R.CO.C ₆ H ₅	1.56	300	30.5	16.5	20.5	0
			400 (a)	4.0	2.5	2.0 (a)	—
615	R.CO.CH ₂ .CO.O.C ₂ H ₅	3.125	75	22.5	11.0	8.0	—
617	R.CO.CHCl ₂	25.0	150	—	17.5	10.0	5.0

TABLE X—continued.

B.R.L. No.	R.X	In vitro Activity μg./ml.	LD50 mg/kg.	Extension in TD50 over Control. Dose Fraction of LD50			
				1/2	1/5	1/10	1/20
625	R.CO.CH ₂ .CH ₂ Cl	6.25	300	—	19.5	17.5	—
629	R.CO.CH ₂ .C ₆ H ₅	12.5	500	22.5	27.5	17.0	11.0
			>500 (a)	13.0	9.0	6.0 (a)	—
			—	—	4.0	0.5 (b)	—
635	R.CO.CCl ₃	25.0	500	—	—	22.5	21.5
643	R.CO.CO.O.C ₂ H ₅	12.5	100	17.5	13.5	10.5	—
649	R.CO-	50.0	200	29.5	12.5	2.5	—
			>1000 (d)	10.0	1.5	0.5 (d)	—
656	R.CO-	6.25	>500	29.5	15.0	11.0	—
			>500 (d)	16.25	5.2	2.75(d)	—
658	R.CO-	0.39	300	—	28.5	26.3	8.5
			500 (a)	—	7.0	0.5 (a)	—
664	R.CO.CH ₂ N-	>50.0	300	15.0	16.0	15.0	—
			1000 (d)	4.0	2.0	3.0 (d)	—
669	R.CO.CH ₂ O-	>50.0	200	—	5.5	2.5	—
672	R.CO.CH(C ₆ H ₅) ₂	>50.0	>500	6.0	1.0	1.0	—
677	R.CO.CH ₂ .S.C ₂ H ₅	6.25	100	—	18.5	9.0	4.0
681	R.CO.[CH ₂] ₁₄ .CH ₃	>50.0	>500	4.0	0.5	—1.0	—
690	R.CO.CH ₂ .NH.CO.O.CH ₂ .C ₆ H ₅	25.0	150	12.5	8.5	1.5	—
710	R.CO.CH.[CH ₂] ₄ .CH ₂	6.25–12.5	>500	—	34.5	23.5	1.5
711	R.CO.[CH ₂] ₂ .CO.CH ₃	25.0	100	—	23.5	—	—
			200 (a)	—	7.0	2.5 (a)	—
			—	—	2.5	2.0 (b)	—
721	R.CO- -CO.R	3.125	>500	—	25.0	17.0	17.0 (e)

TABLE X—continued.

B.R.L. No.	R.X	<i>In vitro</i> Activity $\mu\text{g. ml.}$	LD50 mg kg.	Extension in TD50 over Control. Dose Fraction of LD50			
				1/2	1/5	1/10	1/20
727	$\text{R.CO.CH}_2\text{.NH.CO.C}_6\text{H}_5$	>50.0	400	—	17.0	16.0	1.5 (e)
728	$\text{R.CO}-\text{C}_6\text{H}_4\text{-N=N.C}_6\text{H}_5$	3.125	>500	—	8.0	11.0	2.0 (e)
733	$\text{R.CO}-\text{C}_6\text{H}_4\text{-NH.CO.CH}_3$	3.125–6.25	>500	—	21.0	13.0	5.5 (e)
746	R.CO.O.CH_3	6.25–12.5	50	—	8.5	4.5	—
747	$\text{R.CO.CH}_2\text{-C}_6\text{H}_4\text{-Cl}$	25.0	—	—	12.5	9.0 (e)	—
748	$\text{R.CO.CH(Cl).C}_6\text{H}_5$	25.0	>500	—	2.5	0.5 (e)	—
753	$\text{R.CO.O.C}_4\text{H}_9$	3.125	>500	—	22.0	8.0	—
785	$\text{CH}_2\text{—CH}[\text{CH}_2]_4\text{.S.CO.CH}_3$ 	—	300	—	2.0	2.0	—

to form episulphides the compounds produced have enhanced antitubercular activity. The inactive compounds are shown in Table VIII and the active compounds in Tables IX and X. Since the synthetic method (Doyle, Holland, Mansford, Nayler, and Queen, *J. chem. Soc.*, in the press) was not structurally definite either or both of the preparations, B.R.L. 528 and B.R.L. 599, may be mixtures of the two isomeric structures. Of the compounds in Table IX only 3-mercaptopropylene sulphide (B.R.L. 482) and di(2,3-epithiopropyl) disulphide (B.R.L. 553) show good activity. On the other hand, the majority of episulphides with *S*-acyl groups (Table X) were highly effective chemotherapeutic agents. The compounds with an episulphide ring must, however, be comparatively simple in order to be active; compounds with high activity being confined to *S*-acyl derivatives of 3-mercaptopropylene sulphide (B.R.L. 482). Substitution of methyl groups on any of the carbon atoms almost abolishes activity. The addition of groups other than simple acyl radicals also abolishes activity. The nature of the acyl radical, on the condition that it is a simple group, would not appear to be the main determining factor for

antitubercular activity, but the acetyl (B.R.L. 459) and furoyl (B.R.L. 658) derivatives both have high *in vivo* and *in vitro* activities.

DISCUSSION

A large number of thiol compounds and related derivatives have been investigated for antitubercular activity, and many have proved to be active *in vivo*. There are, however, a number of discrepancies between the *in vitro* activity and the *in vivo* activity, and an explanation for this must be sought.

Since the majority of the episulphides are very active *in vivo*, it would appear that the episulphide structure is the necessary moiety for antitubercular activity. This view is supported by the investigations of Miles and Owen (1952) and Harding and Owen (1954), who have shown that acetylated thiols with a vicinal hydroxyl group in the presence of mild alkali are deacetylated with accompanying ring closure to form episulphides. The activity of the thiols and thiol *O*-esters can therefore be accounted for by such a mechanism. Further confirmation that an active derivative is formed has been obtained by the findings of Mansford and

Langley (to be published), who have shown that the addition of 3-acetylthiopropylene sulphide (B.R.L. 459) to serum leads to the formation of 3-mercaptopropylene sulphide (B.R.L. 482). If B.R.L. 482 is the active derivative, the absence of activity *in vitro* may be explained by the fact that it is an unstable compound and under the mild alkaline conditions of the *in vitro* test it forms inactive polymers.

The activity of the compounds tested is not due to their conversion to ethanethiol which has been shown to be the active agent produced by other thiol antitubercular agents (Oginsky, Solotorovsky, and Brown, 1955, 1956). We have been unable to detect ethanethiol in the expired air of animals injected with any of the compounds tested, nor has it been possible by using B.R.L. 587 containing radioactive ^{35}S to detect ^{35}S in the breath. Over 80% of the injected ^{35}S was recovered in the urine of rats in 24 hr. as sulphate.

The activity of the compounds which are active *in vitro* can possibly be explained by the assumption that the compound is absorbed on the surface of the organisms and the active metabolite (i.e., B.R.L. 482) is formed in close proximity to it and is therefore able to exert an antibacterial action before it is destroyed chemically. While there is little evidence for this theory, it does provide a likely explanation.

This explanation could also account for the apparent discrepancy between activities of 2,3-dimercaptopropyl benzoate (B.R.L. 587) and 1,3-dimercapto-2-propyl benzoate (B.R.L. 680) *in vitro* and *in vivo*. It is possible that B.R.L. 680 can be readily converted *in vitro* to the parent episulphide whereas B.R.L. 587 cannot. *In vivo*, however, the reaction may readily take place, with the result that both compounds are equally active. It is unlikely that the compounds are simply dealkylated to the hydroxyl compound without

ring closure taking place, since the parent hydroxyl compound (B.R.L. 231) of B.R.L. 680 is highly toxic and completely inactive *in vivo*.

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