SUBSTITUTED DITHIOCARBAMATES AND RELATED COMPOUNDS AS TRICHOMONACIDES

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A series of derivatives of dithiocarbamic acid and related compounds have been examined against *Trichomonas vaginalis* and *Trichomonas foetus in vitro* and *in vivo* in the mouse and the rat. The most active compound *in vitro*, sodium dimethyldithiocarbamate, caused a 90 per cent inhibition of growth of *T. vaginalis* and *T. foetus* at 0·07 μg./ml. The *in vitro* activity of the other compounds ranged from 0·17–1·5 μg./ml. There was no direct correlation of *in vitro* and *in vivo* activity. In the assay in mice, sodium 4-morpholinecarbodithioate was the most active compound against *T. vaginalis*. The CD 50 was 3·9 mg./kg. This compound would not cure rats infected intravaginally with *T. vaginalis*. *In vitro* there was an area of non-response in the dose-response curve of *T. vaginalis* to sodium 4-morpholinecarbodithioate and piperidine 1-piperidinecarbodithioate. When mice were infected with *T. vaginalis*, the infections were completely susceptible to treatment with low doses of sodium 1- pyrrolidinecarbodithioate and bis(dimethylthiocarbamoyl) sulphide, but not higher doses. Both of these phenomena, *in vitro* and *in vivo*, may have been due to differences in the solubility of various metal complexes formed by these compounds.

Certain *N*-substituted derivatives of dithiocarbamic acid and of its oxidation product bis(thiocarbamoyl) disulphide are bacteriostatic and fungistatic (Chabrier, Maillard and Quevauviller, 1956; Kaars Sijpesteijn and Janssen, 1959) and inhibit the growth of plant nematodes (Cave, 1958). This report is concerned with the *in vitro* and *in vivo* trichomonacidal properties of these and related compounds.

Experimental

Materials

Trichomonas vaginalis R and *Trichomonas foetus* MSC, previously described (Michaels and Strube, 1961), were used for *in vitro* assays and *in vivo* assays in mice. The C.I. strain of *T. vaginalis* was isolated by us during the course of this work. All strains were maintained in fluid thioglycollate medium with 5 per cent horse-serum (FTM) (Michaels and Strube, 1961).

In vitro *Assays*

The amount of a compound producing growth stasis in 90 per cent of organisms was determined by a two-fold dilution series of the compound involved. Solutions or finely divided suspensions of the compounds in 0·5 ml. of saline (0·9 per cent NaCl, w/v) were added to 4·5 ml. of FTM adjusted to contain an initial population of 10,000 cells/ml.

after this dilution. The highest amount of any chemical tested was 200 μg./ml.

After 22 hr. incubation at 37°, cell populations were determined by direct counts in a Neubauer chamber. The percentages of inhibition were calculated for each level of inhibitor by comparison with untreated controls. The 90 per cent end-points were read from semi-logarithmic plots of these data (Michaels and Strube, 1961).

In vivo *Assays*

Groups of 5 adult, male, albino mice (CF 1 strain) were infected subcutaneously with 200,000, 24 hr. cells in a volume of 0·2 ml. Compounds for treatment of these infections were homogenised in a sterile solution of 5 per cent gelatin. Beginning with the Tolerated Dose, which has been defined as the maximum amount of a drug in mg./kg./day which can be administered for six consecutive days and still allow weight gains comparable to those of the control animals (Michaels and Strube, 1961), drugs were given in serial two-fold dilutions either orally or intraperitoneally. The first treatment was given immediately after infection, and continued once daily for 5 days. Controls were dosed with the suspending medium.

TABLE I

In vitro ACTIVITY OF DERIVATIVES OF DITHIOCARBAMIC ACID AND BIS(THIOCARBAMOYL) SULPHIDE AND DISULPHIDE AGAINST *Trichomonas vaginalis* AND *Trichomonas foetus*

Chemical	Concentration in μg./ml. producing 90 per cent growth inhibition of	
	T. vaginalis	*T. foetus*
4-Morpholinecarbodithioic acid, sodium salt	*	0·31
1-Pyrrolidinecarbodithioic acid, sodium salt	0·64	0·45
1-Piperidinecarbodithioic acid, piperidine salt	*	1·20
Sodium dimethyldithiocarbamate	0·065	0·074
Sodium diethyldithiocarbamate	0·17	0·17
Bis(morpholinothiocarbonyl) disulphide	0·70	0·75
Bis(dimethylthiocarbamoyl) sulphide	0·37	0·31
Bis(dimethylthiocarbamoyl) disulphide	0·36	0·52
Bis(diethylthiocarbamoyl) disulphide	0·72	1·50
Bis(1-piperidinothiocarbonyl) disulphide	0·26	Not tested

* See text for explanation since there was more than one 90 per cent end point for this chemical.

Animals were killed 7 days after infection. The presence of trichomonads in the subcutaneous lesions resulting from injection was verified by microscopic examination of an eosin-stained wet smear. The 50 per cent curative dose (CD 50) was calculated by the method of Reed and Muench (1938). The relative activities of the compounds were obtained by comparing their therapeutic quotients which has been defined as the tolerated dose divided by the CD 50 (Michaels and Strube, 1961).

Compounds were also assayed in spayed, female, albino rats of the Long-Evans strain infected intravaginally with the C.I. strain of *T. vaginalis* (Cavier and Mossion, 1956; Michaels, Peterson and Stahl, 1962). The method of treatment was the same as used for mice except

that the first treatment was not given until at least 1 month after infection. Therapeutic efficacy was evaluated by the presence or absence of the organism in vaginal smears which were made daily. Animals which became negative during the course of treatment and remained so for two additional weeks were considered cured.

RESULTS

Many derivatives of dithiocarbamic acid and of bis(thiocarbamoyl) disulphide were tested which were inactive *in vitro* by the minimal criterion established for this test, i.e., 90 per cent growth inhibition at 200 μg./ml. Sodium dimethyldithiocarbamate was the most active substance tested. It was 90 per cent growth inhibitory to *T. vaginalis* at 0·065 μg./ml. and to *T. foetus* at 0·074 μg./ml. It was the only dithiocarbamate that was distinctly more active than its oxidation products (Table I). All other compounds were also very active against both species of trichomonads and the range of activity was small (0·17–1·5 μg./ml.).

TABLE II

THE *in vitro* DOSE-RESPONSE CURVE OF *Trichomonas vaginalis* TO 4-MORPHOLINECARBODITHIOIC ACID, SODIUM SALT AND 1-PIPERIDINECARBODITHIOIC ACID, PIPERIDINE SALT

Chemical concentration, μg./ml.	Percentage of growth inhibition produced by	
	4-Morpholine-carbodithioic acid, sodium salt	1-Piperidine-carbodithioic acid, piperidine salt
200·0	100·0	97·6
100·0	97·0	98·8
50·0	91·4	99·5
25·0	88·0	98·3
12·5	93·6	73·7
6·2	85·0	89·8
3·1	85·0	85·5
1·6	89·7	91·5
0·8	98·8	75·7
0·4	43·5	58·1
0·2	0·0	0·0

The dose response of *T. vaginalis* to sodium 4-morpholinecarbodithioate and piperidine 1-piperidinecarbodithioate was unusual (Table II). Over the range 1·6–50 μg./ml., increasing the concentration of sodium 4-morpholinecarbodithioate did not necessarily increase the amount of growth inhibition, or increased it only slightly. Thus four, 90 per cent end-points could be calculated at 0·71, 1·6, 7·4 and 37·4 μg./ml. A similar phenomenon was found for piperidine 1-piperidinecarbodithioate over a range of 3·1–25·0 μg./ml. Three, 90 per cent end-points could be calculated, at 1·3, 6·2 and 19·8 μg./ml.

In vivo activity is shown in Tables III and IV. There was no direct correlation of *in vivo* and *in vitro* activity. The compounds which were most active *in vitro*, sodium dimethyldithiocarbamate, sodium diethyldithiocarbamate and bis(1-piperidinothiocarbonyl) disulphide, were not active *in vivo* against *T. vaginalis* and were not tested against *T. foetus*. Piperidine-1-piperidinecarbodithioate and bis(diethylthiocarbamoyl) disulphide were active against *T. vaginalis* and not *T. foetus*. Bis(dimethylthiocarbamoyl) sulphide was much more active against *T. vaginalis* than

T. foetus. With these exceptions, *in vivo* activity was the same against both species of trichomonads. On a weight basis, all the derivatives of bisdithiocarbamic acid were equally active against *T. vaginalis* (average CD 50 about 4 mg./kg., i.p.) except bis(diethylthiocarbamoyl) disulphide which had a CD 50 of 73·7 mg./kg., intraperitoneally. There was a

TABLE III

In vivo EFFICACY OF DERIVATIVES OF DITHIOCARBAMIC ACID AND OF BIS(THIOCARBAMOYL) SULPHIDE AND DISULPHIDE ON EXPERIMENTAL INFECTIONS WITH *Trichomonas vaginalis* IN MICE

Chemical name	Tolerated dose mg./kg.		CD50* in mg./kg./day		Therapeutic quotient**	
	I.P.	Oral	I.P.	Oral	I.P.	Oral
4-Morpholinecarbodithioic acid	100	400	3·9	17·4	25	23
1-Pyrrolidinecarbodithioic acid..	800	800	8·0	20·4	100	39
1-Piperidine carbodithioic acid, piperidine salt	100	100	16·2	141	6·2	0·7
Bis(morpholinothiocarbonyl) disulphide ..	25	200	3·7	9·8	6·8	20·4
Bis(dimethylthiocarbamoyl) sulphide	100	100–200	4·5	8·8	22	11
Bis(dimethylthiocarbamoyl) disulphide ..	12·5–25	+200	4·5	15·6	2·8	12·8
Bis(diethylthiocarbamoyl) disulphide	100	400	73·7	200	1·4	2·0

* Curative dose 50 ** Tolerated dose/CD50

four-fold difference in the range of the CD 50 of derivatives of dithiocarbamic acid (3·9–16·2 mg./kg., i.p.), using *T. vaginalis* as the test organism. Using the criterion of the therapeutic quotient, sodium 4-morpholinecarbodithioate and sodium 1-pyrrolidinecarbodithioate were distinctly more active than any of the other compounds tested against *T. vaginalis*. The former compound also was the most active against *T. foetus*.

TABLE IV

In vivo EFFICACY OF DERIVATIVES OF DITHIOCARBAMIC ACID AND OF BIS(THIOCARBAMOYL) SULPHIDE AND DISULPHIDE ON EXPERIMENTAL INFECTIONS WITH *Trichomonas foetus* IN MICE

Chemical name	Tolerated dose mg./kg.		CD50* in mg./kg./day		Therapeutic quotient**	
	I.P.	Oral	I.P.	Oral	I.P.	Oral
4-Morpholinecarbodithioic acid	100	400	9·3	17·4	10·8	23
1-Pyrrolidinecarbodithioic acid..	800	800	10·4	inactive	76·8	
1-Piperidine carbodithioic acid, piperidine salt	100	100	inactive	not tested		
Bis(morpholinothiocarbonyl)disulphide ..	25	200	6	6·2	4·2	32·2
Bis(dimethylthiocarbamoyl) sulphide	100	100–200	37	67·3	2·7	1·5
Bis(dimethylthiocarbamoyl) disulphide ..	12·5–25	200	8·8	43	1·4	4·6
Bis(diethylthiocarbamoyl) disulphide ..	100	400	inactive	not tested		

* Curative dose 50 ** Tolerated dose/CD50

The *in vivo* dose-response curves of *T. vaginalis* and *T. foetus* to bis-(dimethylthiocarbamoyl) sulphide (Table V) and of *T. vaginalis* to sodium 1-pyrrolidinecarbodithioate (Table VI) were not linear throughout. For comparison, the response of *T. foetus* to sodium 1-pyrrolidinecarbodithioate is also shown in Table VI. It will be recalled that *T.*

foetus was unaffected by this compound when the oral route of treament was used.

Because of the unusual nature of the response to these compounds, the data have been documented as completely as possible. This type of data has never been found with 22 other compounds active systemically in mice against *T. vaginalis* which have been assayed repeatedly by this laboratory. Because of this non-linear response, sodium 1-pyrrolidinecarbodithioate was not assayed in rats.

TABLE V

THE *in vivo* DOSE-RESPONSE CURVES OF *Trichomonas vaginalis* AND *Trichomonas foetus* TO BIS(DIMETHYLTHIOCARBAMOYL) SULPHIDE IN MICE

Dose mg./kg./day	*T. vaginalis*			*T. foetus*	
	i.p.[1]	i.p.	i.p.	i.p.	oral[2]
400·0				0	5
200·0	2[3]	1		1	0
100·0	2	5	1	1	4
50·0	5	5	5	4	2
25·0	5	5	5	3	3
12·5	5	5	5	4	1
6·2	5	5	4	2	0
3·1	0	0	0	2	
1·6	0		0	1	

1. Intraperitoneal route of treatment.
2. Oral route of treatment.
3. Number of animals cured out of a total of 5 treated.

TABLE VI

THE *in vivo* DOSE-RESPONSE CURVES OF *Trichomonas vaginalis* AND *Trichomonas foetus* TO 1-PYRROLIDINECARBODITHIOIC ACID, SODIUM SALT IN MICE

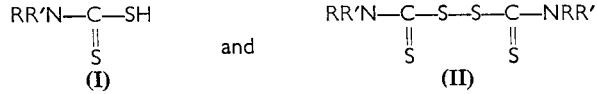
Dose mg./kg./day	*T. vaginalis*		*T. foetus*	
	i.p.[1]	oral[2]	i.p.	oral
800·0	0[3]	1		
400·0			5	
200·0	1	3		0
100·0	3		5	0
50·0	4	5	0	
25·0	5		5	0
12·5	5	1		0
6·2	1		1	0
3·1	0	0		

1. Intraperitoneal route of treatment.
2. Oral route of treatment.
3. Number of animals cured out of a total of 5 treated.

Rats infected intravaginally were treated orally with 12·5–200 mg./kg. (tolerated dose in this species) of sodium 4-morpholinecarbodithioate. The results were negative. As a positive drug control, 2-hydroxy-5-nitropyridine was used (Michaels and Strube, 1961). The CD 50 was 326 mg./kg.

DISCUSSION

The compounds are *N*-substituted derivatives of dithiocarbamic acid (I) and of bis(thiocarbamoyl) disulphide (II):



(I) (II)

That RR'N·CS·S· was the active structure was concluded from the *in vitro* inactivity of *NN*-dimethylthioformamide, *NN*-diethylthioacetamide, thiourea, and rubeanic acid (dithio-oxamide). When RR' was dimethyl or diethyl, the compounds were active; when RR' was dibutyl the compounds were inactive *in vitro*. The morpholino-, pyrrolidino- and piperidino-derivates of dithiocarbamic acid were active. Both the morpholino- and piperidino-derivatives were active in the disulphide series. The pyrrolidino-derivative was not tested.

The *in vitro* order of activity of substituted dithiocarbamates was Me>Et>pyrrolidino>morpholino>piperidino. This is different from the order of activity of these substances as bacteriostatic agents which was morpholino>Me>piperidino (Chabrier, Maillard and Quevauviller, 1956).

It has been found (Kaars Sipjesteijn and Janssen, 1958; 1959) that sodium dimethyldithiocarbamate forms a 1:1 complex with copper at low concentrations, at higher concentrations the 1:2 complex is formed and at still higher concentrations the free ion is present in the medium. The 1:1 complex is relatively soluble, but the 1:2 complex is relatively insoluble. Although all three molecular species are toxic to moulds and bacteria, there is a species variation in sensitivity to the three forms. Since the 1:2 complex is so insoluble, an organism must be extremly sensitive to it to be affected by it. If it is not, there will be growth over the range in which the 1:2 complex is formed. This had been called a zone of inversion growth (Kaars Sijpesteijn, Janssen and van der Kerk, 1957). The response of trichomonads to most of the derivatives of dithiocarbamic acid and of bis(thiocarbamoyl) disulphide was like the response of *Glomerella congulata* to sodium dimethyldithiocarbamate (Kaars Sijpesteijn and Janssen, 1959), i.e., all the organisms were very sensitive at low concentrations of the compounds and there was no zone of inversion growth.

The response of *T. vaginalis* to sodium 4-morpholinecarbodithioate and piperidine 1-piperidinecarbodithioate does not fit exactly any previously described dose-response relationship to these kinds of compounds. Rather than a zone of inversion growth a zone of non-response was found. This zone of non-response was probably due to the formation of an insoluble 1:2 copper complex with the result that the amount of compound in solution increased very slowly over this range.

A real zone of inversion growth was found *in vivo* but to compounds whose *in vitro* response was linear. Whether this was due to chelation with a different metal *in vivo* than *in vitro*, or to an entirely different mechanism, is not known.

Dithiocarbamates and bis(thiocarbamoyl) disulphides were as active *in vitro* against *T. vaginalis* as acinitriazole(2-acetamido-5-nitrothiazole) and 2-amino-5-nitropyridine, more active than 2-amino-5-nitropyrimidine, and less active than the di(4-methyl-3-thiosemicarbazone) of pyruvaldehyde (Michaels and Strube, 1961; Michaels, Peterson and Stahl, 1962). The *in vivo* activity against experimental infections with *T. vaginalis* in mice was equal to or greater than any of the compounds mentioned

excepting the di(4-methyl-3-thiosemicarbazone) of pyruvaldehyde. However, it is not recommended that these compounds be tried clinically since they did not cure vaginal infections in rats and they are extremely toxic (Dubois, Raymund and Hietbrink, 1961).

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