Antithyroid Substances

2. SOME MERCAPTOGLYOXALINES, MERCAPTOTHIAZOLES AND THIOHYDANTOINS

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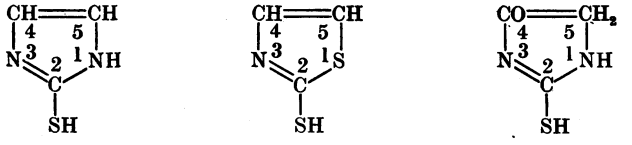
In a previous communication (Searle, Lawson & Hemmings, 1950) we reported on the antithyroid activity of a number of 2-mercaptoglyoxalines, the assays being based on the uptake of ^{131}I by the thyroid gland of rats after a single injection of the drug. We have extended this work to include a further series of 2-mercaptoglyoxalines, several 2-thiohydantoins and a number of 2-mercapto- and 2-amino-thiazole derivatives (Fig. 1). Many of these compounds were recently made accessible as a result of the work of Cook, Heilbron & collaborators (1947–9) and their structural relationship to compounds of known antithyroid activity seemed to us to warrant their investigation from this point of view.



2-Mercaptoglyoxaline 2-Mercaptothiazole 2-Thiohydantoin

Fig. 1.

We are also reporting on the effect of dietary administration over several weeks of some representative compounds of the different groups.

METHODS

The synthesis of new compounds reported on will be described elsewhere.

Assay technique. The screening test was carried out as already described (Searle *et al.* 1950). All drugs were administered by stomach tube, usually at a level of 0·05 mmol./kg. body weight. The dose of radioactive iodide (approx. 1 μc. in 0·5 ml. of 0·9% NaCl without added carrier) was injected intraperitoneally after 1 hr., and the rats killed after a further 4 hr. The ^{131}I uptakes of the thyroids of the dosed and control animals were then compared using a liquid counter, the results being expressed as the percentage depression of the mean ^{131}I uptake of the control animals. The results are summarized in Table 1.

In the longer-term feeding experiments the drugs were mixed at levels of 0·1 and 0·25% with the diet (either crushed cube diet or Glaxo powder diet, code no. RBSS-9). Each group of animals was matched for weight as in the screening tests, and each rat was weighed daily for some days before and during the experimental period. Finally, radioactive iodide was injected as described, and the ^{131}I content of the glands was determined after 24 hr. The results are shown in Table 2.

RESULTS AND DISCUSSION

Mercaptoglyoxalines

2-Mercapto-1-methylglyoxaline has already been reported by Stanley & Astwood (1949) as being 100 times more active than 2-thiouracil in man, and we have found this compound to be among the most active in rats. Jones, Kornfeld, McLaughlin & Anderson (1949) have reported on the antithyroid activity in rats of a number of 1-substituted 2-mercaptoglyoxalines, using the method of Astwood (1943) involving an estimation of compensatory thyroid hyperplasia for the assay. Of the compounds examined, the three most active were 2-mercaptoglyoxaline, and its 1-methyl and 1-ethyl derivatives, these being found to be only half as active as thiouracil. As reported in our previous communication (Searle *et al.* 1950) we found the activity of 2-mercaptoglyoxaline in rats to be about equal to that of thiouracil when tested by the radioactive iodine uptake method.

The only report of the clinical trial of a mercaptoglyoxaline is that of Reveno & Rosenbaum (1950) who successfully treated a small group of hyperthyroid patients with 2-mercapto-1-methylglyoxaline and found the activity of this substance to be about twenty-five times that of propylthiouracil. No toxic reactions were encountered at the dosage reported to be effective. The results of acute toxicity determinations in mice carried out with three representative mercaptoglyoxalines are shown in Table 3. Having in mind the activity of these compounds the figures would indicate that simple

Table 1. *Antithyroid activity of compounds as tested by the single-dose technique in rats*

Compound	Dose (mg./kg.)	No. of rats dosed	Depression of ^{131}I uptake (%)	Significance of differences from control*
2-Mercapto-1-methylglyoxaline	6	7	>93·2	*A*
1-Carbethoxy-2-mercapto-4-methylglyoxaline	9	14	63·4	*A*
2-Mercapto-4:5-diphenylglyoxaline	12·5	7	-2·7	*C*
4-Carbethoxyaminomethyl-2-mercaptoglyoxaline	10	7	-4·6	*C*
4-Carbethoxy-5-hydroxymethyl-2-mercaptoglyoxaline	10	7	16·2	*B*
Di-4:4′-(2-mercaptoglyoxalinyl)methane	10·5	7	-1·1	*C*
1-Carbethoxy-2-mercaptoglyoxaline	0·5	8	7·3	*C*
	2	8	58·8	*A*
	8·5	8	>94·9	*A*
	8·5	7	52·8†	*A*
4-Carbethoxyaminomethyl-1-carbethoxy-2-mercaptoglyoxaline‡	14	7	-48·5	*A*
Di-2:2′-(4-methylglyoxalinyl)disulphide	11	7	23·1	*B*
Di-2:2′-(1-carbethoxy-4-methylglyoxalinyl)disulphide	18·5	7	78·4	*A*
1-Benzoyl-2-thiohydantoin	25	5	93·7	*A*
3-Methyl-2-thiohydantoin	6·5	7	76·4	*A*
5-Methyl-2-thiohydantoin	6·5	9	71·2	*A*
2:4-Dithiohydantoin	7	5	21·1	*B*
5-*iso*Propylidene-2:4-dithiohydantoin	9	5	15·4	*C*
5-Acetamido-2-mercapto-4-*n*-propylthiazole	10	5	2·5	*C*
4-Ethoxymethylene-2-mercaptothiazol-5-one	9	5	-26·5	*B*
5-Amino-4-carbethoxy-2-mercaptothiazole	10	5	4·9	*C*
4-Carbethoxy-5-*cyclo*hexyl-2-mercaptothiazole	14	5	1·8	*C*
5-Acetamido-2-mercaptothiazole	9	5	2·8	*C*
5-Benzylideneamino-2-mercaptothiazole	9	7	-2·3	*C*
2-Mercapto-4:5-benzthiazole	8	5	-26·5	*C*
5-Amino-2-mercaptothiazole	7	5	-2·8	*C*
5-Amino-2-carbethoxythiothiazole	9·5	7	38·8	*A*
2-Mercaptothiazol-5-one	7	6	-33·9	*B*
2-Mercapto-4-*cyclo*pentylidenethiazol-5-one	10	5	25·1	*B*
5-Acetamido-2-carbethoxyaminothiazole	11·5	7	18·2	*C*
2-Benzamido-5-benzoylthioureido-4-phenylthiazole	21	4	29·5	*B*
5-Amino-4-carbethoxy-2-methylaminothiazole	10	5	0·3	*C*
2-Amino-4-carbethoxy-5-methylthiazole	9	5	0·4	*C*
2-Aminothiazole hydrogen maleate	11	6	9·2	*C*
5-Amino-2-benzamidothiazole	11	7	17·5	*C*
4:5:6:7-Tetrahydro-6-methyl-2-methylamino-7-keto-5-thionothiazolo (5:4-*d*)-pyrimidine	11	6	-3·4	*C*
2-Thiobarbituric acid	7	7	-13·5	*C*
Sulphanilylthiourea	12	7	-1·5	*C*
	50	7	14·0	*C*
Sulphanilamide	8·5	6	18·5	*C*
Sulphathiazole	13	7	25·4	*B*
Sulphadiazine	12·5	7	55·3	*A*
p-Hydroxybenzoic acid	100	8	0·8	*C*
Resorcinol	5·5	7	-0·9	*C*

* Probability: $P<0·001$, *A*; $0·1>P>0·001$, *B*; $P>0·1$, *C*.
† 24 hr. uptake experiment.
‡ Some decomposition in HCl.

members of the group are not unduly toxic. Comparable data for 2-thiouracil are also given in Table 3. Dieke, Allen & Richter (1947) report LD_{50} values of 1000 and 2000 mg./kg. for wild and domestic Norwegian rats respectively when 2-thiouracil was given orally.

Introduction of a phenyl group in the 4-position of the mercaptoglyoxaline nucleus gave a reduced antithyroid activity, and the 4:5-diphenyl compound, which had to be administered as a suspension in gum acacia solution owing to its insolubility, proved inactive. This latter finding is in agreement with results already published by Astwood, Bissell & Hughes (1945). The use of 1-carbethoxy-2-mercaptoglyoxaline, in which the imino hydrogen atom is replaced by an easily hydrolysable grouping, failed to prolong the antithyroid action of the glyoxaline as shown by a 24 hr. uptake test with this compound. Somewhat remarkable was the significant increase (48·5%) in radioactive iodine uptake produced by the *N*-carbethoxy derivative of 4-carbethoxyaminomethyl-2-mercaptoglyoxaline. Further investigation with this substance is proceeding. The high activity (78·4% depression) of the disulphide derived from 1-carbethoxy-2-mercapto-4-methylglyoxaline probably indicates a reductive

Table 2. *Effect of antithyroid substances on growth rate and thyroid activity of young rats*

Drug	Level in diet (%)	No. of rats	Duration of dosing (days)	Effect on growth	Thyroid wt. (mg./100 g.) Dosed	Thyroid wt. (mg./100 g.) Controls	Depression of 24 hr. ^{131}I uptake (%)
2-Mercaptoglyoxaline	0·25*	7	9	Immediate arrest of growth	28·9	12·9	>98·1
2-Mercaptoglyoxaline	0·1	7	29	Loss of weight after 9 days	53·2	9·5	97·0
2-Mercapto-4:5-dimethylglyoxaline	0·25*	7	9	Growth slightly reduced	26·8	12·9	43·0
1-Benzoyl-2-thiohydantoin	0·1	3†	29	Loss of weight after 11 days; fits after 15 days	39·2	9·5	—
2-Thiouracil	0·25*	7	12	Slightly reduced growth after 5 days	33·7	12·2	97·5
2-Thiouracil	0·1	8	32	Complete arrest of growth after 10 days	50·4	10·7	—
2-Mercapto-4:5-benzthiazole	0·25*	8	9	Growth slightly reduced	13·5	12·9	−2·7

* In these experiments the drugs were administered mixed with crushed commercial cube diet, this resulting in much less interference with growth than when the Glaxo diet RBSS-9 was employed. This supports the view (private communication from Dr J. Gross) that commercial diets prepared from fishmeal may contain appreciable amounts of thyroxine derived from the gastro-intestinal tract of the fish.

† These three animals died 4–5 days before the controls and other animals were killed. The three other animals in the group had been returned to normal diet (see text).

Table 3. *Acute toxicity of mercaptoglyoxalines in mice*

(LD_{50} values (mg./kg. body weight).)

Drug	Route of injection: Subcutaneous	Intraperitoneal	Intravenous
2-Mercaptoglyoxaline	350	640	1400
4-Ethyl-2-mercaptoglyoxaline	530	570	780
4-Carbethoxy-2-mercaptoglyoxaline	270	116	180
2-Thiouracil	4350	1160	825

fission in the organism to give the corresponding thiol known to be active, this change occurring less readily with the disulphide derived from 2-mercapto-4-methylglyoxaline.

The surprising inactivity of di-4:4′-(2-mercaptoglyoxalinyl)methane may be associated with its low solubility. It does, however, form a soluble sodium salt.

Mercaptothiazoles and aminothiazoles

No 2-mercapto- or 2-amino-thiazole tested showed activity comparable with the most active thiouracils and mercaptoglyoxalines, the largest depression of ^{131}I uptake (38·8%) having been produced with 5-amino-2-carbethoxythiothiazole. Although many derivatives containing this ring system have been examined for antithyroid action, the only ones reported to show appreciable activity in rats are 4-aminophenyl-2′-amino-5′-thiazolyl sulphide ('promizole sulphide') (McGinty & Bywater, 1945), the reduced thiazole, 2-mercaptothiazoline (Stanley & Astwood, 1947) and 3-(phenylaminomethyl)-thiazolidine-2-thione ('T.C. 105') (Rawson, McGinty, Peacock, Merrill, Wilson & Lockhart, 1948). In this connexion, the observation of Stanley & Astwood (1947) that 2-mercapto-4-methylthiazole is as active as thiouracil in humans but not in rats is of interest. Many of the derivatives of this class tested by us were rather unstable, and this may well have had some bearing on the low antithyroid activities observed. The effect of 2-mercapto-4:5-benzthiazole administered to young rats (50 g. wt.) in the diet is shown in Table 2.

Thiohydantoins

Jackman, Klenk, Fishburn, Tullar & Archer (1948) have already reported 5-methyl-2-thiohydantoin to have an antithyroid activity equal to that of thiouracil, but do not mention their method of assay. The three simple hydantoins tested by us had fairly high activity as is shown in Table 1.

The most active derivative reported here, 1-benzoyl-2-thiohydantoin (incorrectly reported as the 1-benzyl compound in Part 1, Searle *et al.* 1950) has also been administered mixed with the diet (Table 2). After 15 days at the level reported, the animals began to have intermittent convulsions soon after they were handled for purposes of weighing, etc. Three animals removed from the group after a further 8 days and given normal diet recovered quickly, but the remaining animals continued to lose weight and died 14 days later. The symptoms observed closely resembled the epileptiform fits described by Chick, El Sadr & Worden (1940) as being produced in rats after 4–5 months on a diet deficient in vitamin B_6. Examination of urine specimens from control and dosed animals for calcium and amino-acid levels showed no appreciable difference.

Miscellaneous compounds

The frequently reported goitrogenic activity of certain sulphonamides led us to examine three members of the group. The most active, sulphadiazine, has already been reported by Stanley & Astwood (1947) to be appreciably less active in humans than in rats. 2-Aminothiazole, active in humans, proved inactive in rats by our test. Our result for sulphanilylthiourea is in accordance with that reported by Astwood *et al.* (1945).

In our test, resorcinol showed no activity, although Doniach & Fraser (1950) found that this substance at the same dosage level, but using the subcutaneous route and shorter time intervals after administration, gave marked depressions of the ^{131}I uptake, in rats.

SUMMARY

A series of 2-mercaptoglyoxalines, 2-thiohydantoins, 2-mercapto- and 2-amino-thiazoles and some miscellaneous compounds has been tested for antithyroid activity in rats.

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