a Stuart-Turner pump in which the parts coming in contact with electrolyte were replaced by similar parts made of Perspex.

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Antithyroid Substances

3. DERIVATIVES OF 2-MERCAPTOGLYOXALINES

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In Parts 1 and 2 (Searle, Lawson & Hemmings, 1950; Searle, Lawson & Morley, 1951) the examination of a range of 2-mercaptoglyoxalines (I), 2-mercaptothiazoles and 2-thiohydantoin for antithyroid activity in young rats was described.

As a result of chemical investigations in the 2-mercaptoglyoxaline series a number of new compounds have been obtained, and these have now been subjected to screening tests for antithyroid activity by the methods already described, namely by determining the effect of a single dose of the drug on the radioactive iodine uptake of the rat's thyroid gland over a 4 hr. period.

With a view to increasing the duration of activity of some antithyroid drugs, particularly the highly active 2-mercapto-1-methylglyoxaline ('methimazole', 'mercazole'), the effect of introducing a hydrolysable grouping on the sulphur atom has been investigated. Results obtained with one such compound, 2-ethoxycarbonylthio-1-methylglyoxaline ('carbimazole', 'neomercazole') (II, $R = OC_2H_5$) were described by Lawson, Rimington & Searle (1951). This substance is at least as active in the rat as its parent thiol, but no evidence was obtained that it was hydrolysed sufficiently slowly *in vivo* to give a markedly increased duration of activity. In clinical use (Doniach, 1953; Poate,

1953), however, it appears to be slightly more active than 2-mercapto-1-methylglyoxaline, and the substance is free of the bitter taste associated with the thiol drugs.

A preliminary account has appeared of the antithyroid activity of some further S-substituted derivatives of 2-mercaptoglyoxalines (Lawson & Searle, 1954). Reaction of 2-mercapto-1-methylglyoxaline with methyl chloroformate, benzyl chloroformate or phosgene yields derivatives (II) which are readily hydrolysed *in vitro* back to the thiol, and which show high antithyroid activity in the rat. Reaction of mercaptoglyoxalines with alkyl halides, on the other hand, yields thioethers (III) which can only be hydrolysed back to the parent thiols under much more drastic conditions.

It is usually assumed that antithyroid agents such as thiourea and the thiouracils and mercaptoglyoxalines owe their activity to the presence of the potentially free thiol group, which by its ready reduction of elementary iodine to iodide ion

interferes with the iodination of tyrosine in the thyroid protein and hence inhibits the synthesis of the thyroid hormone.

2-Alkylthioglyoxalines would therefore be expected to be devoid of antithyroid activity. It was observed by Searle *et al.* (1950), however, that 2-benzylthioglyoxaline showed slight activity, and some 2-methylthio- and 2-benzylthio-glyoxalines have therefore been examined in the present work. A number of them have been found to show low but definite activity.

Ergotamine has been shown to lower the heat-production of the thyroid-fed dog (Bouckaert, 1926) and of the normal rabbit (Marine, Deutch & Cipra, 1927), and has been used in the treatment of hyperthyroidism. It was therefore thought of interest to examine the effect of this drug and of dihydroergotamine on thyroidal iodine uptake by the radioactive iodine technique.

METHODS

The assay technique was similar to that already described (Searle *et al.* 1950).

Rats. Newly weaned hooded rats of Glaxo stock were maintained for some weeks before use on Glaxo powder diet RBSS-9, the low I_2 -content of which results in thyroidal ^{131}I -uptakes of the order of 15–25% over 4 hr. in the control animals. The rats were used in groups of about seven animals, matched for weight and sex. Occasionally batches of these rats did not grow well on the diet, and in some experiments, therefore, rats bred at the Royal Free Hospital School of Medicine have been used.

Drugs. The synthesis of new compounds will be described elsewhere. In the preparation of *S*-methyl derivatives of mercaptoglyoxalines the use of methyl iodide was avoided as any I_2 contamination would cause misleading results.

2-Mercaptohistamine (4,2'-aminoethyl-2-mercaptoglyoxaline) was prepared from butyne-1,4-diol as described by Fraser & Raphael (1952). Attempts to obtain the 1-methyl analogue by condensing the 1,4-diaminobutan-2-one with CH_3NCS instead of KCN were unsuccessful.

Compounds for testing were administered by stomach tube, usually at a level of 0.05 m-mole/kg. body weight in the case of free thiols and *S*-acyl derivatives but at higher levels with *S*-alkyl derivatives. They were given in aqueous solution, as the Na or HCl salts when not otherwise soluble. Ergotamine and dihydroergotamine (Sandoz Products Ltd.) were injected subcutaneously as the tartrate ('Femergin') and methanesulphonate respectively in 0.9% NaCl solution. The control rats were dosed similarly with water or saline solution.

Radioactive iodine. One hour after dosing the drug under test, the rats were injected intraperitoneally with 0.5 ml. 0.9% NaCl, containing ca. 2 μ c of ^{131}I per ml. In some experiments this time interval was increased to 20 hr.

After a further 4 hr. the animals were killed with $CHCl_3$. The thyroids were dissected out, weighed, allowed to stand overnight in 2 ml. 2% NaOH, and dissolved by boiling gently. The solutions were diluted to 10 ml. with water, and the radioactivity in each rat's thyroids was then determined using an M 6 Geiger-Müller liquid counter (20th Century Electronics).

Results obtained are summarized in Table 1.

RESULTS AND DISCUSSION

2-Mercaptoglyoxalines

In previous experiments (Searle *et al.* 1950, 1951) it was found that all substituents in the 4- and 5-positions of 2-mercaptoglyoxaline caused reduced antithyroid activity, the loss of activity being least in the presence of a single 4-alkyl-substituent and almost complete with a strongly polar group, such as free or substituted amino or hydroxyl. The results obtained in the present experiments are in accord with this previous work.

The complete series of 4-alkyl-substituted derivatives from 2-mercaptoglyoxaline to 4-*n*-hexyl-2-mercaptoglyoxaline has now been tested by the same method. By assuming all the compounds to have dose/response curves similar to that of 2-mercaptoglyoxaline (Searle *et al.* 1950), the dose of each which would cause a 50% depression in radioactive iodine uptake can be estimated. Taking the activity of 2-mercaptoglyoxaline as unity, the relative activities of the 4-alkyl derivatives are found to be: methyl, 0.2; ethyl, 0.5; *n*-propyl, 0.5; *n*-butyl, 0.5; *n*-pentyl, 0.2 and *n*-hexyl, 0.15.

Activity thus falls fairly steadily with increasing chain-length, the only anomaly being the 4-methyl derivative. The abnormally low result with this compound might be due to partial oxidation to the inactive 4-carboxylic acid, or to the hyperconjugation effect causing increased electron release into the ring, which seems to be a factor causing reduced antithyroid activity. The introduction of an *iso*-propyl or a 1-formylethyl group into the 1-position of the 4-methyl compound reduced its activity slightly.

The antithyroid activities of some 4-alkyl-substituted mercaptoglyoxalines were estimated using a histological method by Miller & Bergmann (quoted by Jackman, Klenk, Fishburn, Tullar & Archer, 1948). They also found a reduced activity relative to 2-mercaptoglyoxaline in the *isopropyl*, *isobutyl*, *n*-pentyl, *n*-hexyl, *cyclopentyl*, *cyclohexylmethyl* and *benzyl* derivatives, but they found the *n*-propyl compound to be approximately 3 times as active as its parent.

The increased uptake (+48.5%) found in an early experiment with the diethoxycarbonyl derivative of 4-aminomethyl-2-mercaptoglyoxaline (Searle *et al.* 1951) could not be repeated in the present work.

Substances with amino or hydroxyl groups in the 4- and 5-positions were inactive as expected from previous experiments. It had been thought possible that an aldehydic group in these positions would enhance activity, but this was found not to be the case with 4-formyl-2-mercaptoglyoxaline, possibly owing to oxidation to the inactive carboxylic acid.

Table 1. *Effect of various compounds on ¹³¹I-uptake of the rat's thyroid gland over a period of 4 hr.*

Compound	Dose (mg./kg.)	No. of rats dosed	Depression of ¹³¹ I- uptake (%)	Significance of difference from controls*
<i>2-Mercaptoglyoxalines</i>				
2-Mercaptoglyoxaline	1	5	53.8	A
	†(5)	6	2.2)	C
4- <i>n</i> -Butyl-2-mercaptoglyoxaline	0.2	6	-53.8	A
	1	5	35.0	B
	5	6	87.7	A
	8	6	94.2	A
	(8)	6	13.3)	C
4- <i>n</i> -Hexyl-2-mercaptoglyoxaline	9	8	62.4	A
	(9)	6	1.8)	C
4-Benzyl-5-ethyl-2-mercaptoglyoxaline	11	7	9.9	C
4-Ethyl-5- <i>p</i> -hydroxybenzyl-2-mercaptoglyoxaline	12	5	27.8	B
2-2'-Mercaptoglyoxalin-4'-yl propionic acid	8.5	5	19.5	C
2-Mercapto-4-2'-methylthioethylglyoxaline	8.5	8	18.9	C
4- ω -Amino- <i>n</i> -butyl-2-mercaptoglyoxaline	10.5	8	20.0	C
4-4'-Glyoxalinylmethyl-2-mercaptoglyoxaline	9	7	10.0	C
4-Formyl-2-mercaptoglyoxaline	6.5	7	-4.15	C
	50	6	30.5	B
2-Mercaptohistamine	9	6	22.3	C
4-2'-Aminoethyl-2-mercaptoglyoxalin-5-yl acetic acid	10	5	-20.8	C
1-1'-Formylethyl-2-mercapto-4- or 5-methylglyoxaline	8.5	6	43.8	B
	(8.5)	5	5.6)	C
4(5)-Ethoxycarbonylaminomethyl-2-ethoxycarbonylthioglyoxaline	14	6	19.3	B
	30	7	60.5	A
2-Mercapto-4-methyl-1-isopropylglyoxaline	8	8	48.1	A
2-Mercaptoglyoxaline-maleic anhydride adduct	11	8	12.6	C
4- or 5- <i>n</i> -Butyl-1-1'-formyl- <i>n</i> -pentyl-2-mercaptoglyoxaline	13	6	21.1	C
<i>S-Acyl 2-mercaptoglyoxalines</i>				
2-Methoxycarbonylthio-1-methylglyoxaline	0.2	7	57.5	A
	1.0	5	89.0	A
2-Benzoyloxycarbonylthio-1-methylglyoxaline	1	5	86.2	A
	(12)	7	23.8)	C
2-Benzamidomethylcarbonylthio-1-methylglyoxaline	14	6	97.3	A
Di(1-methylglyoxalin-2-yl)dithiolcarbonate, 2HCl	8	5	96.8	A
	14	6	94.7	A
2-Acetylthio-4(5)-methylglyoxaline	11	7	93.0	A
	11	6	71.0	A
<i>S-Alkyl 2-mercaptoglyoxalines</i>				
2-Benzylthioglyoxaline	50	6	38.5	B
	100	6	55.9	A
2-Methylthioglyoxaline	50	6	52.5	B
2-Benzylthio-1-methylglyoxaline, HCl	25	7	41.8	A
	50	6	52.7	B
	(12)	8	11.2)	C
1-Methyl-2-methylthioglyoxaline, HCl	25	7	23.6	B
	50	6	26.0	C
	(8)	7	-4.1)	C
2-Ethoxycarbonylmethylthio-1-methylglyoxaline	10	6	13.8	C
2-Benzylthio-4-methylglyoxaline	50	6	51.8	A
	100	7	74.6	A
4-Methyl-2-methylthioglyoxaline	50	8	48.5	A
4'-Methylthiazolo-(2':3':2:1)-5-methylglyoxaline	7.5	6	13.9	C
	(7.5)	5	2.6)	C
<i>Miscellaneous</i>				
Thiourea	15	7	87.8	A
<i>S</i> -Benzylthiourea, HCl	10	6	27.0	B
	50	7	51.6	B
3:5-Diaminothia-2:4-diazole	6	6	-6.3	C
Ergotamine tartrate	0.5	7	19.7	C
	0.5	12	41.7	B
	1.0	7	24.8	B
	1.0	16	43.8	A
	1.0	18	34.6	A
Dihydroergotamine methanesulphonate	1.0	11	-1.7	C

* The significance of the uptake depression was examined by Student's *t* test, and is expressed as follows; $P < 0.001$, A; $0.1 > P > 0.001$, B; $P > 0.1$, C.

† Parentheses denote those experiments in which ¹³¹I was administered 20 hr. after the drug under test. In all other cases the interval was 1 hr.

It would be interesting to examine a 4-nitro derivative in this connexion, but it has not been found possible to prepare such a compound.

Duration of activity

On increasing the length of the carbon chain in the 4-alkyl-substituted 2-mercaptoglyoxalines, the compounds become increasingly soluble in organic solvents and insoluble in aqueous media. It was thought possible that by virtue of being fat-soluble some higher 4-alkyl-2-mercaptoglyoxalines might have a more prolonged action than the drugs in common use. Moreover, on a molecular basis 4-*n*-butyl-2-mercaptoglyoxaline was the most active 4-substituted mercaptoglyoxaline in the usual radioactive iodine uptake test.

The most satisfactory way of investigating the duration of activity of antithyroid substances would be by a neck-counter method as developed for tests on human subjects by Astwood and co-workers (see, for example, Stanley & Astwood, 1947). Such a method could not be employed in the present work, but it was thought of interest to find the effect of increasing the time interval between administration of the drug and the radioactive iodine from 1 to 20 hr. It was found, however, that at levels of 0.05 m-mole/kg. body weight, 2-mercaptoglyoxaline and also its 4-*n*-butyl and 4-*n*-hexyl derivatives were quite without effect on the uptake of ¹³¹I injected after 20 hr.

S-Acyl-2-mercaptoglyoxalines

On allowing for its greater molecular weight, 2-ethoxycarbonylthio-1-methylglyoxaline was found to have antithyroid activity very similar to that of the parent thiol when tested in the rat by the technique already described at a number of dosage levels (Lawson *et al.* 1951). When the interval between dosing the drug and injecting radioactive iodine was increased from 1 to 20 hr. (see preceding section) the 4 hr. uptake depressions were similarly reduced, and it thus seems that the ethoxycarbonyl derivative is hydrolysed to the thiol too quickly in the rat's body for the duration of antithyroid activity to be markedly prolonged.

High activity, similar to that of the parent 2-mercapto-1-methylglyoxaline, has similarly been found in the following compounds (where R denotes the 2-substituted 1-methylglyoxalanyl radical);

Like the *S*-ethoxycarbonyl derivative, the *S*-benzyloxycarbonyl compound showed no evidence of any prolonged activity in the test with the 20 hr. time interval.

S-Alkyl-2-mercaptoglyoxalines

2-Benzylthioglyoxaline has been examined at higher doses (50 and 100 mg./kg.) than that reported earlier (Searle *et al.* 1950). The *S*-methyl derivative has been examined similarly, as have the *S*-benzyl and *S*-methyl derivatives of 2-mercapto-1-methyl- and 2-mercapto-4-methyl-glyoxaline. At these levels marked depressions of radioactive iodine uptake are obtained, the highest being 75 % following dosage of 2-benzylthio-4-methylglyoxaline at 100 mg./kg.

By utilizing the previously reported results for the free mercaptoglyoxalines at various dose levels it is possible to calculate approximately for each experiment with an *S*-alkyl mercaptoglyoxaline the dose of the corresponding thiol which would give a similar depression in radioactive iodine uptake (Table 2).

The interesting fact emerges that the substitution of a methyl or benzyl grouping on the sulphur atom may produce much less reduction in antithyroid activity in this test than expected. In particular, 2-benzylthio-4-methylglyoxaline has about one-tenth of the activity of the corresponding thiol, and *S*-benzylthiourea hydrochloride one-third to one-eighth of the activity of thiourea. The more active drugs 2-mercaptoglyoxaline and 2-mercapto-1-methylglyoxaline showed a much greater reduction in activity on blocking the sulphur atom, but this seems mainly due to the greater activity of the thiols as the activities of the thioethers are of the same order of magnitude.

The activity of the 2-alkylthioglyoxalines is probably a property of the substances as such and not due to their relationship to the highly active mercaptoglyoxalines. The activity could be due to the reaction of the compounds with iodine in the thyroid gland so that there is a reduced amount of iodine available for thyroid hormone synthesis. Any substance which can reach the thyroid and which can be iodinated can theoretically act in this way, and in the case of resorcinol this mechanism has been demonstrated by the chromatographic identification of iodoresorcinol after incubation of resorcinol with rat-thyroid slices (Fawcett & Kirkwood, 1953). Though in the present work 2-benzylthio-4-methylglyoxaline has been iodinated giving the 5-iodo derivative (m.p. 153–154°, decomp.), reaction of these substances with iodine is complex and pure products are difficult to isolate.

The alternative possibility that the *S*-alkyl derivatives can be converted into thiols in the body was thought unlikely, since *in vitro* this change can only be brought about by boiling with concentrated hydrochloric acid. Moreover, there are no recorded instances of this type of debenzylation *in vivo* (Prof. R. T. Williams, private communication). Fission of the glyoxaline-sulphur link occurs more easily

Table 2. *Antithyroid activity of mercaptoglyoxalines and thiourea compared with that of their S-benzyl and S-methyl derivatives*

Compound	Depression of ¹³¹ I uptake (%)	Dose (mg./kg.)	Approx. dose of thiol to give same depression (mg./kg.)	Ratio: Dose thioether / Dose thiol
<i>2-Mercaptoglyoxaline</i>				
<i>S-Benzyl</i>	39	50	1	50
	56	100	1.5	33
<i>S-Methyl</i>	53	50	1.5	33
<i>2-Mercapto-1-methylglyoxaline</i>				
<i>S-Benzyl hydrochloride</i>	42	25	0.15	170
	53	50	0.2	250
<i>S-Methyl hydrochloride</i>	24	25	0.04	625
	26	50	0.04	1250
<i>S-Ethoxycarbonylmethyl</i>	14	10	0.01	1000
<i>2-Mercapto-4-methylglyoxaline</i>				
<i>S-Benzyl</i>	52	50	5	10
	75	100	8	12
<i>S-Methyl</i>	49	50	5	10
<i>Thiourea</i>				
<i>S-Benzyl hydrochloride</i>	27	10	3	3.3
	52	50	6	8.25

(cf. the ready decomposition of *S*-benzylthiourea by alkali giving benzyl mercaptan).

To check this point, however, 2-mercapto-4-methylglyoxaline and 2-benzylthio-4-methylglyoxaline were administered to rats, and the urine was then examined for the presence of the thiol using 2,6-dichloroquinone chloroimide. At pH 8 this reagent gives with 2-mercapto-4-methylglyoxaline a red-brown product, which is extractable with chloroform. The purplish pink solution so obtained can be used for quantitative determination of the thiol with a Spekker photometer by reference to a calibration curve. This technique has been used by McAllister (1951; McAllister & Howells, 1952) for the analysis of 2-mercapto-1-methylglyoxaline and some thiouracils which give yellow chloroform-soluble products with the reagent.

It was found that following administration of 2-mercapto-4-methylglyoxaline at a level of 50 mg./kg. at least 56% of the dose is excreted in the urine within 18 hr. 2-Benzylthio-4-methylglyoxaline was administered also at a level of 50 mg./kg. The antithyroid effect of this dosage is approximately equivalent to that of 5 mg. of the thiol, and hence one might have expected to find some 2.5–3.0 mg. of thiol in the urine if it were formed from the benzyl derivative. In fact, no thiol was found in the urine, nor when the dosage was increased to 200 mg./kg. In the latter case, a marked odour of benzyl mercaptan was noticed in the urine, which also gave a positive nitroprusside reaction for mercaptan.

A similar experiment was carried out with 2-benzylthio-1-methylglyoxaline hydrochloride (50 mg. of free base/kg.). Again, no thiol was detected in the urine.

Ergotamine and dihydroergotamine

Ergotamine tartrate has been dosed to rats by subcutaneous injection in saline solution, and the effect on radioactive iodine uptake determined in the usual way. Several experiments were carried out, at dose levels of 0.5 and 1.0 mg./kg. body weight.

Radioactive iodine uptakes were markedly depressed, but in some experiments male dosed rats showed much smaller depressions than did the females (e.g., males, 8.2, females, 53.4; males, 5.4, females, 52.8%). This did not always occur, and in the last experiment with ergotamine recorded in Table 1 eight male rats gave a depression of 29.6% and ten female rats a depression of 32.8%. (These values are calculated from control rats of the same sex only.)

Results with ergotamine have been more erratic than with the various antithyroid drug derivatives examined, and the sex difference sometimes found could not be linked with any factor such as the age of the rats or the time of year.

Dihydroergotamine methanesulphonate had no effect on the uptakes of either male or female rats.

SUMMARY

1. A further range of substituted 2-mercaptoglyoxalines has been tested for antithyroid activity in rats.

2. All 4-substituted 2-mercaptoglyoxalines are less active than 2-mercaptoglyoxaline, the most active being the *n*-butyl derivative.

3. *S*-Acyl derivatives of active 2-mercaptoglyoxalines are also active.

4. *S*-Alkyl derivatives of some mercaptoglyoxalines show weak activity.

5. Ergotamine administration reduces thyroidal iodine uptake, a sex difference sometimes being observed.

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The Branched-Chain Fatty Acids of Butterfat

6. FURTHER INVESTIGATIONS ON THE C₁₅ SATURATED ACIDS

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In work previously reported (+)-12-methyltetradecanoic and 13-methyltetradecanoic acids were isolated from mutton fat (Hansen, Shorland & Cooke, 1953), and from butterfat (Hansen, Shorland & Cooke, 1954*a*), while *n*-pentadecanoic acid was isolated from mutton fat (Hansen, Shorland & Cooke, 1954*b*). As, however, the isolation procedure in each case involved hydrogenation, it was not established with certainty that these acids were originally present in the fats as saturated constituents.

In this paper it is shown that without hydrogenation, and by use of a highly efficient fractionating column, two fractions were obtained from butterfat, the first of which contained mainly a mixture of the methyl esters of (+)-12-methyltetradecanoic and 13-methyltetradecanoic acids and the second consisted essentially of methyl *n*-pentadecanoate.

EXPERIMENTAL

Optical rotations were measured on the pure liquids without the use of solvents.

Preliminary fractionation. The butterfat used in this work was supplied by the Rangitaiki Plains Dairy Co. Ltd., Whakatane. The crude methyl esters (51.620 g.) prepared from the butterfat were heated on the water bath under the

vacuum of a water pump to remove traces of water and solvents which were unavoidably contaminated with a small proportion of the more volatile esters. The residue was distilled in a falling-film still at approximately 2.5 mm. and 135° yielding distillate (V1 + V2, 7323 g.) of which the more volatile part (V1, 372 g.) was collected in a dry-ice trap. The residue R1 (44584 g.) was redistilled in a falling-film still at 130° in high vacuum to give a distillate (V3, 42453 g.) and a residue (R2, 1841 g.).

V3 (15889 g.) was distilled *in vacuo* in a Vigreux column (210 × 6 cm.) and the first fraction redistilled in another Vigreux column (480 × 4.5 cm.). From the latter fractionation, fraction 1,6 the saponification equivalent of which approached that of pentadecanoic acid, was selected for the investigation reported in this paper. This concentrate was refractionated in column *G* patterned on column *E* (Shorland, 1952) (cf. Table 1). Column *G* (250 × 3.5 cm. with 3.5 mm. diameter coil) when tested with a 1:1 mixture of heptane-methylcyclohexane at atmospheric pressure was found to possess more than ninety theoretical plates.

C₁₅ Fractions. Fractions 1,6,1 to 1,6,3 were not further examined, as they appeared to consist essentially of methyl myristate. The remaining fractions corresponded in saponification equivalent to methyl esters of C₁₅ acids. Fractions 1,6,4–1,6,6 which were optically active and possessed low melting points, were combined and denoted A, while fraction 1,6,7 with relatively high melting point and with no detectable optical rotation was denoted B.