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Insect Chemosterilants. 1,2,4-Dithiazolium Salts and Related Compounds as Additives to Housefly Diet

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A number of 1,2,4-dithiazolium salts and 2,4-dithiobiurets sterilize houseflies when fed as a diet additive. Only 3,5-bis(dialkylamino)-1,2,4-dithiazolium salts were active against male flies, but some less highly substituted analogs and a number of dithiobiurets were effective against mixed sexes. The results are compared to those obtained by injecting many of the compounds directly into the flies.

We recently reported that a number of substituted diamino-1,2,4-dithiazolium salts (Chang *et al.*, 1972; Oliver *et al.*, 1972b) and similarly substituted 2,4-dithiobiurets (Oliver *et al.*, 1971) are sterilants of male houseflies, *Musca domestica* L. The activities of the compounds against female flies, however, were not studied except for the observation that 3,5-bis(dimethylamino)-1,2,4-dithiazolium chloride sterilized mixed sexes of houseflies at substantially lower doses than those required to sterilize the males (Fye *et al.*, 1969). Furthermore, most of the male sterilants in the former reports were tested by injecting them into the flies' bodies. This is a useful technique for quantitative laboratory studies, but not one that could easily be adapted to the sterilization of thousands of millions of insects. Therefore, to determine the effects of these chemosterilants on both sexes, and to compare the results of the injection and feeding techniques, we tested these compounds as additives to the diet of adult houseflies. We have synthesized and screened well over a hundred dithiobiurets, dithiazolium salts, and related compounds, but only compounds that were active are included in Tables I and II; a few examples of inactive compounds will be mentioned in the text to illustrate the effects of varying structure upon biological activity.

Biological Tests. Details of the procedure were described previously (Fye *et al.*, 1966). Briefly, each compound was added on a w/w basis to a diet of sucrose, nonfat dry milk, and powdered egg yolk (6:6:1). Flies that were kept on the medicated diets were allowed to mate and their reproductive performance was evaluated and compared with that of control flies. To assess the effects on males, the treated males were crossed with virgin untreated females and the fertility of the mated females was again evaluated. The concentrations of the compounds were decreased in successive tests from 1% to levels causing less than 50% sterility, but only the lowest concentration causing 95–100% in one of the dietary media was recorded as the "sterilizing concentration." See Supplementary Material Available paragraph at the end of article.

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EXPERIMENTAL SECTION

Physical data have been reported for most of the dithiazolium salts (Oliver *et al.*, 1972a,b) and dithiobiurets (Oliver *et al.*, 1971). Previously undescribed compounds were prepared as follows.

3,5-Bis(dimethylamino)-1,2,4-dithiazolium Iodide (2). This compound was obtained by oxidizing 1,1,5,5-tetramethyl-2-4-dithiobiuret (**23**) with iodine in absolute ethanol, mp 263° dec (from acetonitrile).

Anal. Calcd for $C_6H_{12}IN_3S_2$: C, 22.72; H, 3.81; N, 13.25; S, 20.22. Found: C, 22.74; H, 3.68; N, 13.31; S, 20.21.

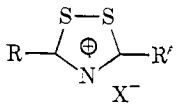
3-(Dimethylamino)-5-[(2-hydroxyethyl)methylamino]-1,2,4-dithiazolium Iodide (5). A mixture of potassium thiocyanate (10 g) and dimethylthiocarbamoyl chloride (12.4 g) in acetone (115 ml) was refluxed for 0.5 hr, cooled, and filtered. The filtrate was treated dropwise with *N*-methylethanolamine (7.5 g); then the resulting solution was evaporated and the residue was dissolved in absolute ethanol (60 ml). A solution of iodine (12.7 g) in absolute ethanol (100 ml) was added dropwise to the stirred solution. The iodide salt separated and was collected by filtration and recrystallized from absolute ethanol to give 10.5 g of **5**, mp 181–183°.

Anal. Calcd for $C_7H_{14}IN_3OS_2$: C, 24.21; H, 4.06; N, 12.10; S, 18.47. Found: C, 24.07; H, 4.02; N, 12.23; S, 18.21.

3-(Cyclohexylamino)-5-(dimethylamino)-1,2,4-dithiazolium Bromide (18). A mixture of potassium thiocyanate (0.2 mol) and dimethylthiocarbamoyl chloride (0.2 mol) in acetonitrile (200 ml) was refluxed for 15 min, cooled, and filtered. The filtrate was cooled and stirred and treated dropwise with cyclohexylamine (0.2 mol), 48% hydrobromic acid (0.2 mol), and 30% hydrogen peroxide (0.2 mol), in that order. Filtration provided 15.7 g of **18**, mp 237–240° dec, and a second crop was obtained by concentrating the filtrate and adding acetone (31.9 g, mp 243–245° dec, total yield 74%). Recrystallization from 95% ethanol gave pure **18**, mp 249–251° dec.

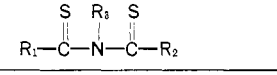
Anal. Calcd for $C_{10}H_{17}BrN_3S_2$: C, 37.04; H, 5.59; N, 12.96. Found: C, 37.28; H, 5.58; N, 13.09.

Table I. Lowest Sterilizing Concentrations of Substituted 1,2,4-Dithiazolium Salts Fed to Houseflies



Compd no.	ENT no.	R	R′	X	Concentration, % Treated ♂	Treated ♀ ♂
1	62112	NMe_2	NMe_2	HSO_4	0.25	0.01
2	62228	NMe_2	NMe_2	I	0.025	0.025
3	62120	NMe_2	NMeEt	I		0.01
4	62077	NMe_2	NEt_2	HSO_4		0.1
5	62206	NMe_2	$NMeCH_2CH_2OH$	I	0.05	0.025
6	62208	NMe_2	$N(CH_2CH_2OH)_2$	I		0.05
7	62207	NMe_2	$NMeCH_2CH_2$-2-pyridyl	I		0.05
8	62271	NMe_2	1-Pyrrolidinyl	Br		0.01
9	62226	NMe_2	Piperidino	Br	1.0	0.025
10	62078	NMe_2	Morpholino	HSO_4		0.1
11	62436	NMe_2	4-Methyl-1-piperazinyl	ClO_4HClO_4	0.025	0.005
12	62179	NEt_2	NEt_2	HSO_4		0.5
13	62118	1-Pyrrolidinyl	1-Pyrrolidinyl	I		0.05
14	62162	1-Pyrrolidinyl	Piperidino	HSO_4		0.5
15	62163	1-Pyrrolidinyl	Morpholino	HSO_4		0.05
16	62161	Morpholino	Morpholino	HSO_4		0.01
17	62110	NMe_2	NHMe	HSO_4		1.0
18	62609	NMe_2	NH-cyclohexyl	Br		0.5
19	62117	NMe_2	NH-1-adamantyl	HSO_4		0.5
20	62558	NMe_2	$NHNMe_2$	Br		1.0
21	52538	NMe_2	$=CC(CH_3):NC(CH_3):CH$	$HClO_4$		1.0
22	62605	NH_2	SCH_3	CH_3SO_4		0.1

Table II. Lowest Sterilizing Concentrations of Substituted 2,4-Dithiobiurets Fed to Houseflies



Compd no.	ENT no.	R_1	R_2	R_3	Concentration, %, treated ♀ ♂
23	62230	NMe_2	NMe_2	H	0.05
24	62158	NMe_2	NEt_2	H	0.25
25	62227	NMe_2	$N(CH_2CH_2OH)_2$	H	1.0
26	62159	NMe_2	Piperidino	H	0.5
27	62160	NMe_2	Morpholino	H	0.25
28	62134	NMe_2	NMe(phenyl)	H	0.025
29	62246	Pyrrolidinyl	Pyrrolidinyl	H	0.25
30	62273	Pyrrolidinyl	Piperidino	H	0.5
31	62180	Pyrrolidinyl	Morpholino	H	0.1
32	62229	Piperidino	Morpholino	H	0.5
33	62205	Morpholino	Morpholino	H	0.25
34	62244	NMe_2	NH_2	H	1.0
35	62252	NMe_2	NHMe	H	0.5
36	62248	NMe_2	NHEt	H	1.0
37	62245	NMe_2	NH-1-adamantyl	H	0.5
38	62567	NMe_2	NMe_2	NMe_2	0.25
39	62291	$Me_2NCSN=C(SMe)NMe_2$			0.25

5-(Dimethylamino)-3*H*-1,2,4-dithiazol-3-one Dimethylhydrazone Hydrobromide (20). The procedure was the same as for the synthesis of **18** except that 1,1-dimethylhydrazine was used in place of cyclohexylamine. Crude **20** was precipitated from the acetonitrile solution by adding ether and was recrystallized from ethyl acetate–acetic acid (64%, mp 225–227° dec).

Anal. Calcd for $C_6H_{13}BrN_4S_2$: C, 25.27; H, 4.59; N, 19.64. Found: C, 25.41; H, 4.59; N, 19.53.

5-(Dimethylamino)-3-(2,5-dimethyl-3*H*-pyrrol-1-ylidene)-3*H*-1,2,4-dithiazole Hydrogen Perchlorate (21). A mixture of 5-(dimethylamino)-3-(methylthio)-1,2,4-dithiazolium perchlorate (6.0 g, Oliver *et al.*, 1972a) and 2,5-dimethylpyrrole (2.0 g) in glacial acetic acid (250 ml) was slowly heated until the boiling point was just reached, and then the solution was immediately filtered. Upon cooling **21** crystallized from the filtrate (4.1 g, 60%), mp 227–29° dec. An analytical sample was recrystallized from acetic acid, mp 235° dec.

Anal. Calcd for $C_{10}H_{14}ClN_3O_4S_2$: C, 35.34; H, 4.15; N, 12.36. Found: C, 35.40; H, 4.13; N, 12.28.

3-Imino-5-(methylthio)-3*H*-1,2,4-dithiazole Methyl Sulfate (22). Isoperthiocyanic acid (15 g, Seltzer and Considine, 1970) and dimethyl sulfate (45 ml) were heated together until a clear solution resulted. The solution was cooled and a little methanol was added, followed by ethyl acetate in several portions to precipitate the product (21.6 g, 78%, mp 112–114°). Recrystallization from acetonitrile gave pure **22**, mp 112–115°.

Anal. Calcd for $C_4H_8N_2O_4S_4$: C, 17.38; H, 2.92; N, 10.14; S, 46.41. Found: C, 17.21; H, 2.87; N, 10.02; S, 46.61.

RESULTS AND DISCUSSION

Figure 1 illustrates the relationship between the dithiobiurets (I) and the diamino-1,2,4-dithiazolium salts (II). Dithiobiurets are the most common synthetic precursors of the diamino dithiazolium salts (Diveley, 1965; Oliver *et al.*, 1972, 1972b), and the parallel sterilizing activities of the two classes suggested to us that the active species was probably the same whether a dithiobiuret or the corresponding dithiazolium salt was administered (Chang *et al.*, 1972). The other two types of dithiazolium salts, III and IV, were synthesized as described previously (Oliver *et al.*, 1972b).

Effects of Dithiazolium Salts on Mixed Sexes. These results resemble those previously obtained by injection of male flies in that the most active dithiazolium salts were those that had both exocyclic nitrogen atoms fully substituted (compounds **1–16**, Table I). Because the feeding technique for testing chemosterilants is not quantitative, occasional discrepancies occur such as the apparent differences between the activities of **1** and **2**. Nevertheless, the general order of activity could be determined. For exam-

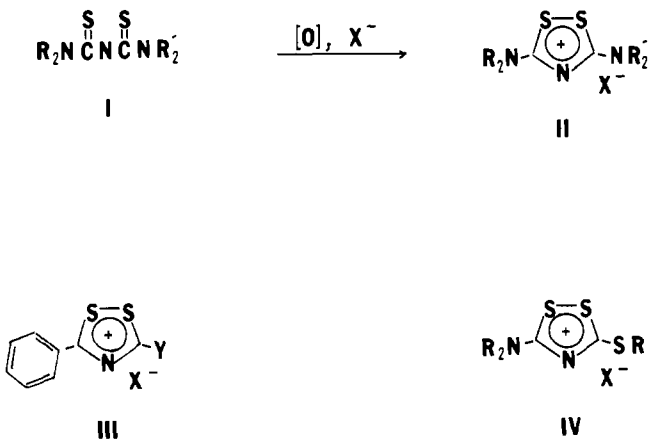


Figure 1. Dithiobiurets and dithiazolium salts.

ple, within this series (1–16), dimethylamino and pyrrolidinyl substitution provided somewhat higher activity than morpholino groups, which in turn were somewhat superior to diethylamino and piperidino groups. The most highly active dithiazolium salt, a *N*-methylpiperazine derivative **11**, was not included in the previous study and is being investigated further.

An interesting development was the activity of some trisubstituted derivatives, **17–20**; no compounds of this type were active against males in the injection tests. However, the number of active compounds of this type seems to be very limited; none of a series of dimethylamino, anilino, or substituted anilino, dithiazolium salts were active, and various other salts related to **17–20** (derived from other primary amines and hydrazines) were inactive or nearly inactive. Similarly, salts **21** and **22** represent the first active compounds in this series that do not have amino substituents at both the 3 and 5 positions. But again these examples appear to be exceptions to the general rule; none of several 3-phenyldithiazolium salts (III, Y = dialkylamino or alkylthio) were active, and a number of 3-(dialkylamino)-5-(alkylthio)-1,2,4-dithiazolium perchlorates (IV) were also inactive.

Effects of Dithiazolium Salts on Male Flies. Comparison of male sterility results from the feeding *vs.* injection techniques reveals marked differences. Dithiazolium salts **8, 9, 10, 15,** and **16** (Table I), which were highly active by injection, were inactive or nearly so in the feeding experiments. Consistent with the injection results, compounds **17–22** were inactive when males only were fed these compounds.

Effects of Dithiobiurets on Mixed Sexes. These results also tended to parallel those obtained previously by injecting the compounds into males and, as before, the structural requirements for activity were very similar to those observed for the dithiazolium salts. In general, dithiazolium salts were more active than the corresponding dithiobiurets; for example, **6, 9,** and **10** (Table I) were more active than **25, 26,** and **27** (Table II), respectively. The comparison is not really reliable, however, since the tetrasubstituted dithiobiurets decompose upon contact with air. Unsymmetrical dithiobiurets can disproportionate into mixtures of symmetrical and unsymmetrical dithiobiurets (Oliver *et al.*, 1971); possibly the surprisingly high activity of **28** is in part due to such a disproportionation since the corresponding dithiazolium salt was entirely inactive. Further decomposition of the dithiobiurets also occurs, and most of the compounds in Table II were probably more or less complex mixtures by the time they were ingested by the flies. The air oxidation of 1,1,5,5-tetramethyl-2,4-dithiobiuret has been studied in some detail (Chang and Oliver, 1972; Oliver and Stokes, 1972), and it was found that indeed a dithiazolium salt was the major decomposition product. A few di- and trisubstituted dithiobiurets (**34–37**) were active in these tests; only tetrasubstituted dithiobiurets were active in the injection tests (Oliver *et al.*, 1971). In contrast to their tetrasubstituted analogs, the di- and trisubstituted dithiobiurets are easily handled and stored without decomposition.

The last two compounds in Table II may merit additional comment. The 3-dimethylamino derivative **38** (Zielinski, 1969) is the first 3-substituted dithiobiuret to show any activity; 1,1,5,5-tetramethyl-3-phenyldithiobiuret, for example, was inactive. It is interesting that **38** is in the same oxidation state as the dithiazolium salts, and could, in principle, cyclize to one of the latter (addition of HX followed by the loss of dimethylamine).

The *S*-methyl derivative **39** was prepared by methylation of **23**; **39** was active in these feeding experiments as well as against male flies by injection. We previously pointed out that the reactivity of **39** toward nucleophiles should resemble that of the dithiazolium salts, and postulated that this might provide an explanation for the activity of this compound (Oliver *et al.*, 1972b).

Effects of Dithiobiurets on Male Flies. In contrast to the results obtained by injection (Oliver *et al.*, 1971), the dithiobiurets in Table II were essentially inactive when males only were fed these compounds. This is quite surprising, particularly since these samples had already decomposed to mixtures that presumably contained significant quantities of the corresponding dithiazolium salts, many of which were active. Other sulfur-containing compounds are also formed (Oliver and Stokes, 1972); possibly the male flies found these mixtures too unpalatable to consume sufficient dosages of the chemicals.

In conclusion, both male and female houseflies can be sterilized by selected dithiazolium salts and dithiobiurets when the compounds are administered as dietary supplements. Only 3,5-bis(dialkylamino)dithiazolium salts are active against males; the same compounds are the most effective ones against females as well, but some less highly substituted analogs and several 2,4-dithiobiurets also show significant activity. Small, compact amino substituents provide greater activity than larger ones—for example, dimethylamino and pyrrolidinyl derivatives are more highly active than diethylamino or piperidino derivatives.

Supplementary Material Available. Detailed results of screening all compounds mentioned in this article will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 20 × reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N. W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JAFC-73-753.

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