

Synthesis and Insect Growth Regulating Activity of Thiosemicarbazones of Methyl 2-Pyridyl Ketones

Lars-Erik K. Pedersen, Axel Svendsen and Per D. Klemmensen

Research Department, Cheminova A/S, PO Box 9, DK-7620 Lemvig, Denmark

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Thirty-six thiosemicarbazones of methyl 2-pyridyl ketone and of some other methyl ketones were tested on *Dysdercus cingulatus*, *Drosophila melanogaster*, *Locusta migratoria*, *Aedes aegypti*, *Tenebrio molitor* and *Musca domestica*. Several derivatives were toxic, delaying or preventing ecdysis. The structure-activity relationship is discussed. The mode of action of these compounds appears to be different from that of juvenile hormones, anti-juvenile hormones, or chitin biosynthesis inhibitors.

1. Introduction

Previously, several thiosemicarbazones have been found to possess a wide spectrum of biological activities. Thus, antiplasmodial,¹ anti-tumour,² anti-trypanosomal,³ anti-inflammatory,⁴ anti-viral,⁵ and bactericidal⁶ activities have been reported. Thiosemicarbazones also show activity as chemosterilants on houseflies (*Musca domestica*),⁷ screw worms (*Cochliomyia hominivorax*),⁸ and boll weevils (*Anthonomus grandis*).⁹

Recently a number of methyl 2-pyridyl ketone thiosemicarbazones have been reported to be effective as toxicants and ecdysis inhibitors on milkweed bugs (*Oncopeltus fasciatus*).^{10,11} The effect of compound I (Table 1) has been correlated with a delayed and erratic production of ecdysteroids^{12,13} and discussed in relation to eclosion hormone release, but the exact mode of action still remains obscure.¹³

The present paper describes the synthesis and the insecticidal effects, including the insect growth regulatory properties, of a series of thiosemicarbazones on various insect species; the structure activity relationship is discussed.

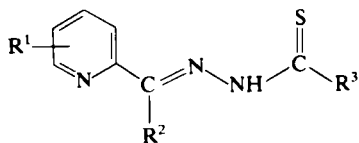
2. Experimental

2.1. Synthesis of compounds

Thiosemicarbazones were prepared by (a) the condensation of equimolar quantities of a thiosemicarbazide and an aldehyde or ketone, or (b) the treatment of a methyl hydrazonecarbo-dithioate with the appropriate amine as previously described.^{11,14,15} References for known compounds are given in Tables 1-3. The synthesis of the substituted methyl 2-pyridyl ketones needed for the preparation of the new thiosemicarbazones IV-IX (Table 1) will be reported elsewhere.

¹H-Nuclear magnetic resonance (n.m.r.) spectra were recorded on a JEOL PMX60 spectrometer operating at 60 MHz, with tetramethylsilane ($\delta=0.00$) as internal standard. All the compounds exhibited ¹H-n.m.r. spectra consistent with the proposed structures. The n.m.r. spectra of most of the thiosemicarbazones were complicated by the presence of various proportions of the (E) and (Z) forms. This finding is in agreement with a previous investigation of geometrical isomerism in pyridine-2-carbaldehyde thiosemicarbazones,¹⁶ which were also shown to exist in (E) and (Z) forms. In the dry crystalline state, they were stable for several

Table 1. Structure and physical data of thiosemicarbazones with the general structure



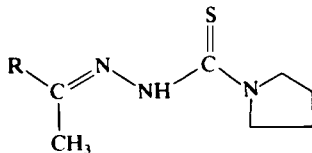
Compound	Structure			M.p. (°C)	Lit. m.p. (°C)	Reference
	R¹	R²	R³			
I	H	Methyl	Pyrrolidin-1-yl	151–152	147–148	14
II	H	H	Pyrrolidin-1-yl	181–184	193–194	17
III	H	Isopropyl	Pyrrolidin-1-yl	157–158.5	161–163	11
IV	5-Ethyl	Methyl	Pyrrolidin-1-yl	72–74		
V	3,5-Dichloro	Methyl	Pyrrolidin-1-yl	116–119		
VI	4-Chloro	Methyl	Pyrrolidin-1-yl	160–161		
VII	5-Butyl	Methyl	Pyrrolidin-1-yl	98–99		
VIII	5-Methoxy	Methyl	Pyrrolidin-1-yl	163–164		
IX	5-Phenoxy	Methyl	Pyrrolidin-1-yl	134–135.5		
X	H	Methyl	Piperidin-1-yl	157–160	152–153	14
XI	H	Methyl	Dimethylamino	155–157	155–156	14
XII	H	Methyl	Ethylamino	130–133	131.5–133	18
XIII	H	Methyl	Allylamino	110–112	107–108	15
XIV	H	Methyl	Benzylamino	149–151	141–143	15
XV	H	Methyl	Diethylamino	118–121.5	115–116	14
XVI	H	Methyl	Diocetylamino	— ^a		
XVII	H	Methyl	Morpholino	191–193 ^b	186–188 ^b	11

^a Low melting solid.^b With decomposition.

days, whereas in solution in several protic and aprotic solvents, they were labile and in time were converted to a mixture of the geometrical isomers.

The purity of all compounds was checked by thin-layer chromatography (t.l.c.) on silica gel plates eluted with mixtures of methanol and dichloromethane, and by high-performance liquid

Table 2. Structure and physical data of thiosemicarbazones with the general formula



Compound	Structure R	M.p. (°C) or n_D at 25°C	Lit. m.p. (°C)	Reference
XVIII	2-Furyl	162–165	165.5–169	11
XIX	Phenyl	138–140	137.5–138.5	11
XX	4-Chlorophenyl	158–160		
XXI	2-Thienyl	152–153	150–151	11
XXII	Pyrazin-2-yl	175–176	181.5–183	11
XXIII	Isopropyl	179–181		
XXIV	Pentyl	1.5440		
XXV	3-Pyridyl	149–151	149.5–150	11
XXVI	4-Pyridyl	182–185	185–185.5	11
XXVII	Ethoxycarbonyl	82.5–84		
XXVIII	Dimethoxymethyl	1.5668		
XXIX	Methylthiomethyl	1.6209		
XXX	3-Methylbut-2-enyl	65–68		

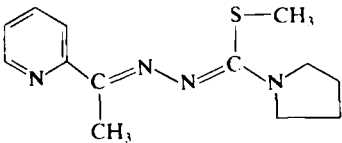
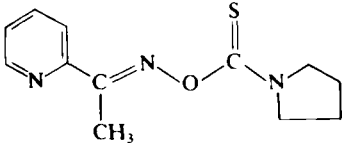
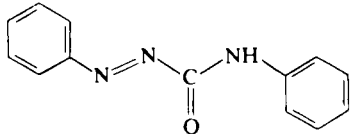
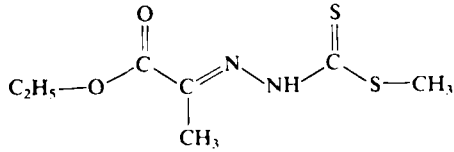
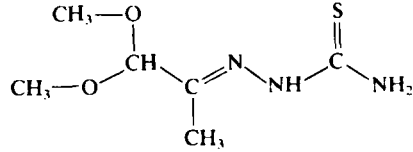
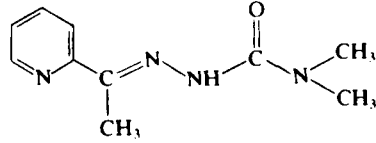
chromatography on a HP 1084 B Liquid Chromatograph by elution with mixtures of a 0.03M phosphate buffer (pH 7) and acetonitrile.

2.1.1. Methyl 2-pyridyl ketone *O*-(pyrrolidin-1-ylthiocarbonyl)oxime (compound XXXII)

To an ice-cooled solution of thiocarbonyl chloride (1.26 g; 11 mmol) in dichloromethane (10 ml) was added a solution of methyl 2-pyridyl ketone oxime (1.43 g; 11 mmol) and triethylamine (1.13 g; 11 mmol) in dichloromethane, over a period of 45 min. Then a mixture of pyrrolidine (0.91 g; 13 mmol) and triethylamine (1.30 g; 13 mmol) in dichloromethane (10 ml) was added over a period of 15 min. After a further 30 min, the reaction mixture was extracted twice with water (20 ml); the extract was dried with anhydrous sodium sulphate, and the solvent was evaporated to leave a brown sticky mass. Column chromatography on silica gel (40 g), eluted with methanol+dichloromethane (2.5+97.5 by volume), gave 1.80 g of crystalline material.

Recrystallisation from ethyl acetate gave the title compound as colourless needles, m.p.

Table 3. Miscellaneous compounds studied

Compound	Structure	M.p. (°C) or n_D at 25°C	Lit. m.p. (°C)	Reference
XXXI		1.6596		
XXXII		109-110		
XXXIII		115-116	116-117	19
XXXIV		80.5-83		
XXXV		128-129	127-130	20
XXXVI		225-227		

109–110°C; n.m.r. (deuteriochloroform): 1.73–2.37 (4H, m), 2.50 (3H, s), 3.53–4.07 (4H, m), 7.13–7.43 (1H, m), 7.53–7.88 (1H, m), 8.03–8.29 (1H, m), 8.50–8.70 (1H, m).

2.1.2. Methyl 2-pyridyl ketone 3-methyl-4,4-(tetramethylene)thiosemicarbazone (compound XXXI)

Sodium hydride (0.30 g of a 55% suspension in mineral oil; 6.6 mmol) was washed free of mineral oil with three portions of light petroleum (5 ml of each) and suspended in dry dimethylformamide (10 ml). To the suspension was added a solution of the thiosemicarbazone I (1.49 g; 6 mmol) in dimethylformamide (6 ml) at ambient temperature. When the evolution of hydrogen gas had subsided, neat iodomethane (1.42 g; 10 mmol) was added in one portion to the clear orange solution, and the reaction mixture was stirred at ambient temperature for 1 h, during which the colour was gradually discharged. The mixture was poured into water (150 ml) and extracted with diethyl ether (2×40 ml).

The combined ether extracts were dried with anhydrous sodium sulphate, and the solvent was evaporated to leave a yellow oil containing some dimethylformamide, which was removed by kugelrohr distillation to give the title compound (1.41 g; 90%) in 98% purity, as shown by gas–liquid chromatography (g.l.c.); n_D at 25°C 1.6596; u.v. (ethanol) $\lambda_{\max}$ (log $\epsilon_{\max}$); 330 nm (3.84); n.m.r. (deuteriochloroform): 1.73–2.09 (4H, m), 2.50 (3H, s), 2.53 (3H, s), 3.46–3.84 (4H, m), 7.01–7.36 (1H, m), 7.48–7.88 (1H, m), 8.24 (1H, br d), $J=8$ Hz), 8.50–8.77 (1H, m).

2.1.3. 1,1-Dimethoxyacetone 4,4-(tetramethylene)thiosemicarbazone (compound XXVIII)

A mixture of 1,1-dimethoxyacetone (0.59 g; 5 mmol) and 4,4-(tetramethylene)thiosemicarbazide (0.73 g; 5 mmol) was refluxed in methanol (20 ml) for 2 h, after which, t.l.c. with methanol+dichloromethane (5+95 by volume) exhibited one major spot (R_F 0.85). Evaporation of the solvent and column chromatography on silica gel (15 g) eluted with methanol+dichloromethane (3+97 by volume) gave the title compound as a viscous yellow oil: n_D at 25°C 1.5668; n.m.r. (deuteriochloroform): 1.87 (3H, s), 1.78–2.30 (4H, m), 3.40 (6H, s), 3.72–4.10 (4H, m), 4.59 (1H, s) 8.37 (1H, br s).

2.1.4. 4-Methylpent-3-en-2-one 4,4-(tetramethylene)thiosemicarbazone (compound XXX)

A mixture of 4-methylpent-3-en-2-one (4.32 g; 44 mmol) and 4,4-(tetramethylene)thiosemicarbazide (2.90 g; 20 mmol) was refluxed in acetonitrile (100 ml) containing catalytic amounts of acetic acid and potassium acetate. After 4 h, the reaction mixture was poured into water (800 ml) and extracted with dichloromethane (2×50 ml). The combined extracts were dried with anhydrous sodium sulphate, and the solvent was evaporated to leave a yellow oil (4.70 g).

Column chromatography on silica gel (60 g) eluted with ethyl acetate+hexane (1+9 by vol) gave the title compound (1.00 g) as yellow crystals: m.p. 65–68°C; n.m.r. (deuteriochloroform): 1.63–2.33 (13H, m), 3.59–4.01 (4H, m), 5.53–6.20 (1H, m), 9.61 (1H, br s).

2.1.5. Ethyl pyruvate 4,4-(tetramethylene)thiosemicarbazone (compound XXVII)

A mixture of ethyl pyruvate (1.28 g; 11 mmol) and 4,4-(tetramethylene)thiosemicarbazide (1.45 g; 10 mmol) in 96% ethanol containing catalytic amounts of acetic acid and potassium acetate was refluxed for 6 h. The solvent was evaporated to leave a viscous yellow oil, which crystallised after being left at room temperature overnight.

Recrystallisation from propan-2-ol gave the title compound (1.30 g, 53%) as light yellow crystals: m.p. 82.5–84°C; n.m.r. (deuteriochloroform): [1.33 (t, $J=7.5$ Hz) and 1.38 (t, $J=7.5$ Hz), 3H together], 1.66–2.41 (4H, m), [2.10 (s) and 2.24 (s), 3H together], 3.55–4.08 (4H, m), [4.29 (q, $J=7.5$ Hz) and 4.33 (q, $J=7.5$ Hz), 2H together], 8.68 (1H, br s).

2.1.6. 1-(Methylthio)acetone 4,4-(tetramethylene)thiosemicarbazone (compound XXIX)

Under conditions identical to those described above (section 2.1.5.) 1-(methylthio)acetone (1.16 g; 11 mmol) and 4,4-(tetramethylene)thiosemicarbazide (1.45 g; 10 mmol) gave a yellow oil. Column chromatography on silica gel (25 g) eluted with dichloromethane gave the title

compound (1.75 g; 75%) as a yellow oil: n_D at 25°C 1.6209; n.m.r. (deuteriochloroform): 1.70–2.29 (10H, m), 3.28 (2H, s), 3.55–4.12 (4H, m), 8.42 (1H, br s).

2.1.7. Methyl 3-(1-ethoxycarbonylethylidene)hydrazinecarbodithioate (compound XXXIV)

A mixture of ethyl pyruvate (6.00 g; 51.6 mmol) and methyl hydrazinecarbodithioate (4.80 g; 39.4 mmol) was refluxed in methanol (40 ml) containing a catalytic amount of acetic acid until the solution turned clear. The reaction mixture was cooled overnight; the precipitate was filtered off, washed with cold methanol and air-dried to give the title compound (6.90 g; 79%) as light yellow needles: m.p. 80.5–84.5°C; n.m.r. (deuteriochloroform) 1.41 (3H, t, $J=7$ Hz), 2.27 (3H, s), 2.68 (3H, s), 4.45 (2H, q, $J=7$ Hz), 13.63 (1H, br s).

2.2. Insect tests

The effects of the compounds were assayed on the following species.

2.2.1. Houseflies (*Musca domestica* L.), fruitflies (*Drosophila melanogaster* Meig.), and yellow mealworms (*Tenebrio molitor* L.)

Tests on these species in the adult stage,²¹ all stages,²¹ and the pupal stage,²² respectively, were carried out as previously described.

2.2.2. Cotton stainers (*Dysdercus cingulatus* F.)

Ten last-instar nymphs, less than 24 h old, were used for each dose. Before and during the experiments, the insects were kept at 27°C and 75% r.h., under a 14-h photoperiod. During experiments, the cotton stainers had free access to water and cotton seeds. Chemicals were applied topically as acetone, or acetone+dimethyl sulphoxide (4+1 by vol) solutions to the thorax (2 μ l per insect) of bugs anaesthetised with carbon dioxide/diethyl ether. The insects were inspected daily until moulting, death or termination of the experiments.

2.2.3. African migratory locusts (*Locusta migratoria* Reiche and Fairmaire)

Five to ten penultimate-instar nymphs, less than 24 h old, from a crowded colony were used for each dose. The insects were kept at 30°C and 25% r.h., under a 12-h photoperiod. During experiments, the insects had free access to fresh young barley leaves and wheat bran. Chemicals were applied as described above, except that 4–8 μ l of solution per insect were used. The insects were inspected daily until moulting, death or termination of the experiment.

2.2.4. Yellow fever mosquitoes (*Aedes aegypti* L.)

Twenty last-instar mosquito larvae were used for each test concentration. Acetone solutions (200 μ l) were mixed with 100 ml tap water 1 h before the larvae were added, and the insects were then kept at 27°C, under a 14-h photoperiod, and fed with 'TetraMin' flakes. The larvae were kept under daily observation until pupation, death or termination of the experiment.

2.3. Light microscopy

The structure of the cuticle from the hind femora of *L. migratoria* was subjected to light microscopy analysis by a standard method.²³ Fourth-instar treated nymphs were taken at a time when control locusts moulted into fifth-instar nymphs. In addition, newly hatched adult locusts were treated with the test compound and killed 9 days later, when the femora were sectioned for microscopy.

3. Results

In general, the solubility of the compounds was low. To test high doses it was therefore necessary to apply suspensions rather than solutions to the insects, which made the actual dose level less precisely defined.

A summary of the results from the insecticidal screening appears in Table 4. The tested

compounds showed varying levels of potency against the different insect species used. At effective doses, the treated insects showed no morphological signs of ecdysis at the time the control insects moulted, but several days later they died, still without visible signs of moulting. Very few insects (apart from the diflubenzuron-treated reference individuals) died during unsuccessful moults.

At low doses, a considerable number of the cotton stainers moulted, but 20–90% of the adults that resulted had slight morphological defects, most of which could be described as creased apical parts of the wings. An experiment using compound I at effective and sub-effective doses was performed on cotton stainers that had been pre-treated with piperonyl butoxide (1 µg per nymph), but no enhancement of any effect was observed.

Table 4. Insecticidal effects of the compounds on four insect species

Compound	<i>Dysdercus cingulatus</i> ^a (nymphs)		<i>Drosophila melanogaster</i> ^b (all instars)		<i>Locusta migratoria</i> ^c (nymphs)		<i>Aedes aegypti</i> ^d (larvae)	
I	+++	DE	+		++	DE	0	DE
II	0		+		0		+	
III	0		0		0		0	
IV	++	DE	+		+	DE	++	DE
V	++	DE	0		0		++	DE
VI	+		+		0		++	DE
VII	++	DE	0		0		+	DE
VIII	+	DE	+		0		++	DE
IX	0		0		0		+++	
X	0		+		0		+	
XI	++	DE	+		++	DE	++	DE
XII	+	DE	++		+		0	DE
XIII	0		++		0		0	
XIV	0		+		0		++	DE
XV	+++	DE	0		0		++	DE
XVI	++	DE	0		0		0	
XVII	+		0		0		0	
XVIII	+	DE	0		0		0	
XIX	+	DE	0		0		0	
XX	+		0		0		+	
XXI	+	DE	0		0		0	
XXII	++	DE	0		0		++	DE
XXIII	+		0		0		0	
XXIV	+		0		0		0	
XXV	0		0		0		0	
XXVI	+		0		0		+	
XXVII	+++	DE	0		0		0	
XXVIII	+	DE	0		+		0	DE
XXIX	+		0		0		+	
XXX	++	DE	0		0		0	
XXXI	++	DE	0		0		0	
XXXII	0		0		0		0	
XXXIII	+		+		0		+	DE
XXXIV	+		0		0		0	
XXXV	+		0		0		0	
XXXVI	0		0		0		0	
Diflubenzuron	+++		+++		++++		++++	
control	0		0		0		0	

^a +, ++, and +++ = more than 50% mortality at 100, 10 and 1 µg per insect, respectively.

^b +, ++, and +++ = more than 50% mortality at 100, 10 and 1 mg kg⁻¹ of larva medium, respectively.

^c +, ++, +++ and ++++ = more than 50% mortality at approximately 500, 100, 10 and 1 µg per insect, respectively.

^d +, ++, +++ and ++++ = more than 50% mortality at 10, 1, 0.1 and 0.01 mg litre⁻¹ of water, respectively.

0 = less than 50% mortality at the highest dose for each insect.

DE = delay of ecdysis.

Light microscopy investigations of the cuticles from eight individual fourth-instar locust nymphs, treated with high doses (100–500 µg per insect) of compound I, revealed no abnormal development. Even if the insects had made no visible attempt to moult, they had formed a new cuticle under the old one. Examination of sections from five adult individuals, treated with 600 µg of compound I per locust, revealed no effect on chitin deposition (as evaluated from the thickness of the endocuticle) or any effect on the sclerotisation in the mesocuticle. At doses of compound I that produced a few moults in locusts, all the emerging fifth-instar nymphs were bleeding from the base of the legs, and these insects eventually died.

Compounds XXVII and XXX caused a peculiar but clearly dose-dependent lethargy in cotton stainers. After treatment, the bugs moved very slowly and became unresponsive to touching of the antennae with a brush. The effect was limited to the cotton stainers, and the ED₅₀ for this effect was approximately 1.5 µg per insect.

In addition to the tests mentioned in Table 4, the compounds were tested on pupae of the yellow meal worm *T. molitor*, but none of the compounds showed any significant effect at 10–100 µg per pupae.

On adult houseflies *M. domestica*, only compound IV showed some toxicity (LD₅₀=9 µg per fly), while none of the other compounds proved toxic to houseflies.

4. Discussion

Taking compound I as a starting point, it appears that changing the substitution position in the pyridine ring (compounds XXV and XXVI) resulted in a significant decrease of the biological activity. This observation corresponds with recent reports of the effect on the milkweed bug *Oncopeltus fasciatus*, and of the antimalarial activity.^{11,24} The results from the tests on *Dys. cingulatus*, and to some extent on *Dros. melanogaster* and *L. migratoria*, of compounds with various substituents on the pyridine ring, indicate that the 5-ethyl, 3,5-dichloro and 5-butyl derivatives (compounds IV, V and VII) were fairly active, but none of them was as active as compound I.

The 4-chloro, 5-methoxy and 5-phenoxy substituted compounds (VI, VII and IX) all showed good activity against *A. aegypti*. Compound IX which was by far the most active member of the whole series on *A. aegypti*, was devoid of activity against the other species. As no prolongation of the treated instar has been observed, and taking the narrow activity spectrum into consideration, it seems reasonable to suggest that the mode of action of this compound is different from that of the others.

Substitution of the methyl group in the R₂ position of the thiosemicarbazone chain (Table 1) by hydrogen (II) or by an isopropyl group (III) resulted in reduced activity. S-Methylation (XXXI) also resulted in decreased activity. These findings are in accordance with the results obtained on *O. fasciatus*.¹¹

Variations in the R₃ substituents (compounds X–XVII in Table 1) revealed that diakylamino derivatives, especially compound XV, were as active as the best pyrrolidin-1-yl derivatives on *Dys. cingulatus*. The morpholino analogue XVII caused no delay of ecdysis and showed only weak toxicity against *Dys. cingulatus*. The effects corresponded fairly well to that of the piperidin-1-yl analogue (X). Interestingly, compound XVII showed only very low activity on *O. fasciatus*, while compound X was very active on this species.¹¹ None of the previously published oxo-analogues, or compound XXXVI (semicarbazones), were effective against insects. The most effective pyridine-based compounds are the structures I, XI and XV.

The activities of the non-pyridine analogues from Tables 2 and 3 were generally moderate, and few of the compounds affected species other than *Dys. cingulatus*. The highest activity was shown by compound XXVII. The pyrazin-2-yl compound XXII had some effect on *A. aegypti* but, surprisingly, did not have the same activity spectrum as the corresponding 2-pyridyl compound (I). Two open chain structures XXXIV and XXXV showed some toxicity on *Dys. cingulatus*, but as no inhibition of ecdysis was observed, it is suggested that the mode of action of these compounds is different.

It appears from Table 4 that few of the compounds have an activity spectrum covering all four insect species. However, the active compounds **I**, **IV** and **XI** affect all of the four test species and may therefore be suspected to possess a fairly general insecticidal activity. It is interesting to note that the higher alkyl analogues **XV** and **XVI**, derived from compound **XI**, do not possess a comparable activity spectrum.

The reported effects and levels of activities of these structurally varied thiosemicarbazones do not correspond to any category of well known or commercial insect growth regulating compounds. The lack of effect on *T. molitor* pupae, as well as the lack of production of nymphal-adult intermediates of *Dys. cingulatus*, suggests strongly that the effects observed were not due to juvenile hormone activity. Likewise the *L. migratoria* and the *Dros. melanogaster* tests revealed no anti-juvenile hormone effect such as the formation of precocious adults and minipupae.

The microscopic analysis seems to rule out a significant effect on chitin biosynthesis and sclerotisation, but the emergence of bleeding locusts indicates that some defects in the integument can occur. As the insects treated with thiosemicarbazones made no attempt to moult, the effect was distinct from that of the chitin biosynthesis inhibitor diflubenzuron, which causes death during the actual ecdysis.

Two classes of compounds, azasteroids and some non-steroidal secondary and tertiary amines, have been reported to cause at least partly, the same type of activity as described here for the thiosemicarbazones.²⁵⁻²⁷ On *A. aegypti*, *Manduca sexta* and other insect species, these compounds are known to disrupt the moulting cycle, partly by preventing ecdysis. They have also been demonstrated to block the conversion of phytosterols into ecdysteroids in insects, but at present, it is not clear whether this effect is the sole cause of the inhibition of the insect development.

Although **I** has been shown to cause aberrant ecdysteroid titres in the haemolymph of *O. fasciatus*, the reported non-curative effect of ecdysteroid injections into thiosemicarbazone-treated individuals again indicates a mode of action other than the inhibition of moulting hormone biosynthesis.¹⁰ A hypothesis has been put forward that the effects observed are mediated via inhibition of eclosion hormone release. Except for the demonstrated fluctuations in the ecdysteroid titre,^{10,13} which might lead to an aberrant eclosion hormone release, no evidence has as yet been presented to support this hypothesis.

It has been reported that certain thiosemicarbazones block ribonucleoside diphosphate reductase (an enzyme that catalyses a critical and possibly rate-limiting step in DNA synthesis and cell division) from rat tumour cells.^{28,29} This report, and the effects on insects described here, might suggest that moult inhibition can be caused by interference with fundamental processes that have no direct relation to ecdysis.

However, the finding that new cuticle is formed in spite of treatment with thiosemicarbazones indicates that blocking of specific ecdysis regulating systems cannot be ruled out. The lethargy-inducing effect of compounds **XXVII** and **XXX** may be explained by a fairly general antimetabolic activity of these two compounds.

Nothing is known of the metabolism in insects of the compounds studied, but the effects described here show no potentiation by pretreatment with piperonyl butoxide, which might suggest that mixed-function oxidases do not play a key role in the metabolism of **I** in *Dys. cingulatus*. Although the activity level of the thiosemicarbazones is lower than that of commercial insecticides and insect growth regulators, the present study indicates that further work on this class of compounds may prove fruitful. As the moulting processes are crucial for insect development, further investigation of chemical interference with ecdysis may lead to the development of new specific insecticides.

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