

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO	
RAD: 12-9988- -4	FECHA: 2013-07-16 19:39:44
DEP: 2020 DIRECCION DE NUEVAS CREACIONES	EVE: 378 FASE NACIONAL INCLUIDO CAPITULO I
TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II	FOLIOS: 5
ACT: 519 PRESENTACION	

DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado(a)/Señor(a)
LUZ CLEMENCIA SUAREZ DE PAEZ

Asunto: Radicación: 12-9988- -4
 Trámite: 11
 Evento: 378
 Actuación: 519
 Oficio: 12637
 Folios: 5

Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	X	Capítulo I	X	Capítulo II	
No. Solicitud Internacional	PCT/US2010/044525				
Fecha de Presentación Internacional	05/08/2010				

1. Documentos estudiados

Descripción:	Radicado N°12-009988-00000-00000	24/Enero/2012
Reivindicaciones:	Radicado N°12-009988-00000-00000	24/Enero/2012
Prioridad:	US 61/232,152	07/Agosto/2009

2. Análisis de la Prioridad

Se acepta la reivindicación de Prioridad porque esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

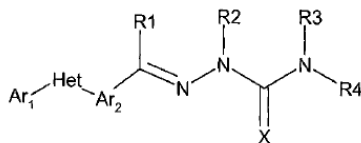
De acuerdo con lo establecido en el numeral 1.2.2.2 del capítulo primero del Título 10 de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por el solicitante al momento de comenzar el examen para las reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada.

Para el caso de la presente solicitud, al verificar la tasa pagada, todo el ultimo capitulo reivindicatorio presentado se tendrá en cuenta para realizar el examen de patentabilidad.

Título de la solicitud: “Composiciones Plaguicidas”.

El problema planteado en esta solicitud es la necesidad de desarrollar compuestos plaguicidas que posean nuevos modos de acción.

Para resolver este problema técnico la solicitud en estudio proporciona un compuesto



de la fórmula donde los radicales están debidamente definidos (radicado N°12-009988-00000-00000, Pág. 4 – 10) que actúa como plaguicida.

El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A 01 N 43/64.

4. Excepción a la Patentabilidad (Art 20 D 486 literal (c) y (d))

El objeto de la reivindicación 13 y 15 no es patentable, puesto que por un lado menciona las semillas y estas hacen parte de lo citado como plantas y por otro lado se menciona un método de tratamiento en animales, según el Art mencionado.

5. Reivindicaciones relativas a un uso o a un segundo uso (Art 14 y 21 D 486)

Los procesos de las reivindicaciones 8, 9 y 14 corresponden al uso del compuesto, mencionado en la reivindicación 1 para controlar la plaga. Es evidente que las llamadas etapas de “aplicar la molécula... para controlar una plaga” y “aplicar una molécula... a una planta genéticamente modificada...” corresponden a la forma de usar los compuestos de la reivindicación 1, aunque en su preámbulo se reclame como proceso. Se le aclara al solicitante que un proceso cuya única etapa corresponde a una aplicación, dicha aplicación es un uso.

Por lo tanto dicho proceso no es patentable de acuerdo con el artículo 14.

6. De la Solicitud de Patente

Título

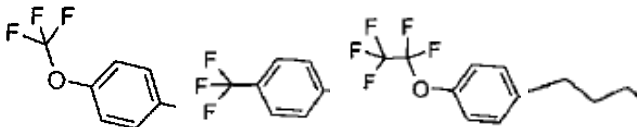
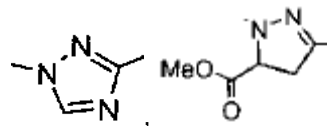
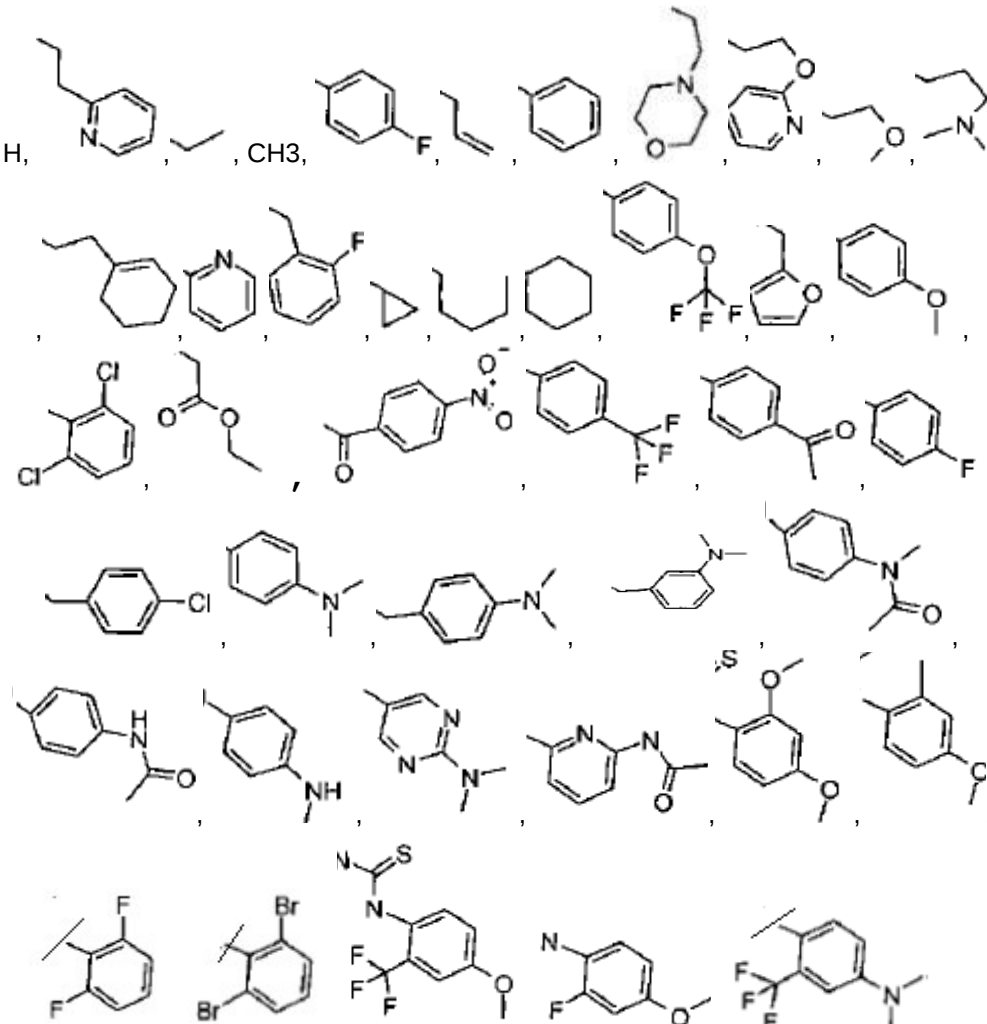
El título no corresponde con el objeto de la solicitud porque el término “plaguicida” es muy general puesto que hace referencia a muchos compuestos, por lo tanto se debe especificar al nombre de la estructura general mencionada en los ejemplos, por lo cual se sugiere al Solicitante hacer la corrección necesaria.

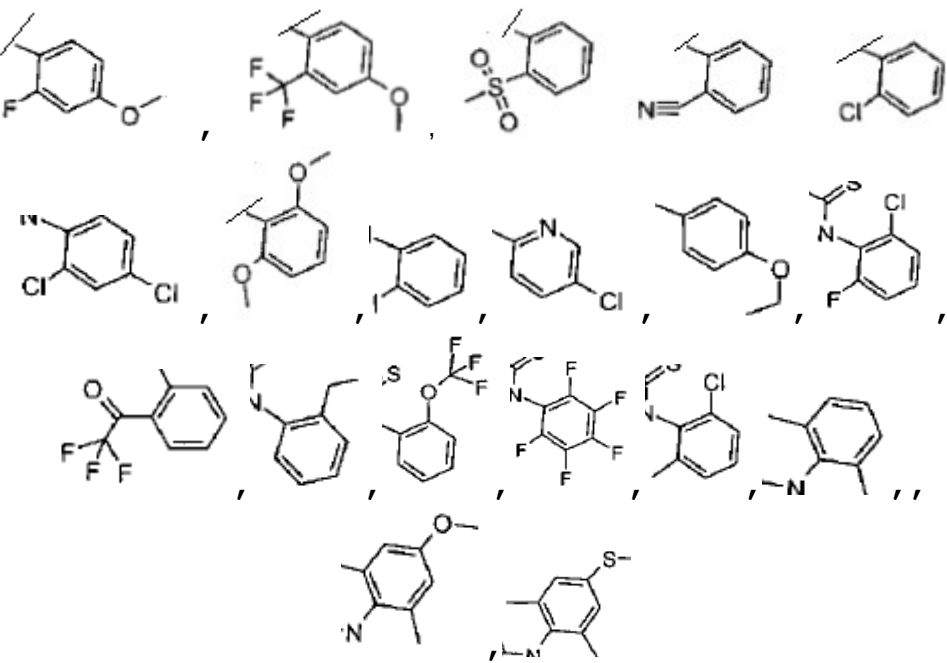
Reivindicaciones (Art 30 D 486, Art 22 PCT)

La reivindicación 1 no se encuentra debidamente soportadas en la descripción puesto que en las realizaciones preferidas hace referencia aparentemente a la preparación y actividad de los compuestos mencionados en la tabla 1, puesto que no se ilustra allí

todos los ejemplos soportados, lo cual no evidencia la actividad de todos los compuestos sino que se hace una generalización de manera especulativa. Se sugiere restringir la solicitud a una razonable cantidad de acuerdo al tipo de compuestos que ha sido sintetizado y probado, es decir, que se encuentran debidamente soportados en el capítulo descriptivo. Teniendo en cuenta la descripción en los ejemplos se encuentra:

Tabla 1. Ejemplos de los compuestos soportados

Sustituciones	Soporte de los ejemplos presentados
Ar ₁	
Het	
Ar ₂	
X	S
R1	H, CH ₃
R2	H
R3 o R4	

Sustituciones	Soporte de los ejemplos presentados
	
R3 y R4 forman	

El solicitante debe definir específicamente la materia y el alcance de la invención a través de las características técnicas esenciales que se encuentren enteramente soportadas en la descripción.

La reivindicación 1 no es concisa, porque presenta una excesiva complejidad puesto que cada uno de los radicales abarca una cantidad excesiva de definiciones de las cuales no existe una gran proporción que muestre actividad, lo cual dificulta determinar la extensión del objeto que se busca proteger.

La reivindicación 10 menciona un derivado del compuesto de interés, lo cual no se encuentra debidamente sustentado puesto que en las realizaciones preferidas no se evidencia la actividad de dichos derivados. Por lo tanto la actividad de dichos compuesto seria especulativa. El solicitante debe definir específicamente la materia y el alcance de la invención a través de las características técnicas esenciales que se encuentren enteramente soportadas en la descripción

La reivindicación 11 menciona que en la molécula el hidrógeno y el carbono pueden ser reemplazados por sus respectivos isotopos pero la actividad de dichos compuestos no se encuentra debidamente sustentada en las realizaciones preferidas. El solicitante debe definir específicamente la materia y el alcance de la invención a través de las características técnicas esenciales que se encuentren enteramente soportadas en la descripción

La reivindicación 12 hace referencia a una composición pero no se mencionan los componentes y las proporciones de los mismos. Aunque no se encuentra debidamente sustentada puesto que en las realizaciones preferidas no se menciona la actividad de dichas composiciones con sus respectivas proporciones.

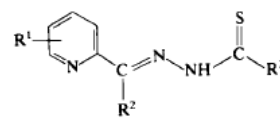
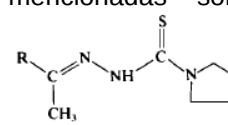
7. Determinación del Estado de la Técnica

El Estado de la Técnica se determinó con base en la Fecha de Presentación de la Solicitud de Prioridad, Agosto 07 de 2009 y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación
D1	US2002019370	"1,2,4-triazole based compounds that can be used as insecticides or acaricides and processes"	14/Febrero/2002
D2	Lars-Erik et Al, Pesticide Science, Dinamarca, vol 25, p 462 - 470	"Synthesis and Insect Growth Regulating Activity of Thiosemicarbazones of Methyl 2-Pyridyl Ketones"	1984

El documento D1 ^[1] divulga compuestos de fórmula (I) para el control de insectos y ácaros, donde los radicales Ar, R¹, Q y R₂ están debidamente definidos (Pág.1, Párr. [0006] a Pág.2, Párr. [0019]).

El documento D2 ^[2] divulga la síntesis y los efectos insecticidas de las tiosemicarbazonas, incluyendo las propiedades reguladoras del crecimiento de insectos. Donde las estructuras de tiosemicarbazonas

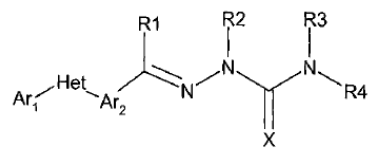
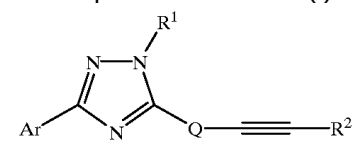
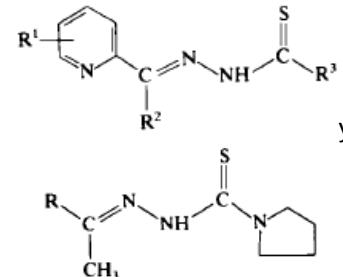
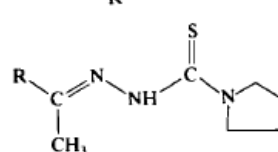
mencionadas son las siguientes estructuras generales  y la estructura , con sus radicales debidamente definidos (Págs. 462 y 463, tabla 1 y 2).

8. Examen de Patentabilidad (Art 14 D486)

Nivel Inventivo (Art18 D486)

La **reivindicación 1** consiste en una molécula, donde los sustituyentes están debidamente definidos (Rad. N° 12-009988-00000-0000 Pág. 162)

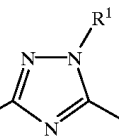
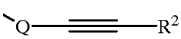
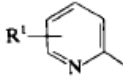
Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características esenciales	D1	D2
Compuesto de formula 	Compuesto de formula(I) 	 y 
Ar ₁ :fenilo, piridilo, entre otros sustituido por H, F, Cl Br, I, CN, alquilo C ₁ -C ₆ , haloalquilo C ₁ -C ₆ , alcoxi C ₁ - C ₆ , entre otros	Ar. fenil o piridil sustituido por H, halógeno, haloalquilo, alquil, entre otros	No se menciona

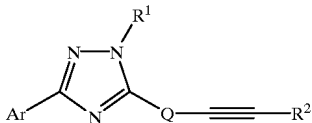
¹URL: [http://worldwide.espacenet.com/publicationDetails/biblio?](http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=20020214&CC=US&NR=2002019370A1&KC=A1)

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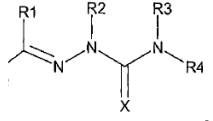
²Anexo 1, artículo científico

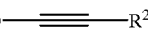
Het: anillo heterocíclico, saturado o insaturado de 5 o 6 miembros, que contiene uno o mas heteroátomos seleccionados de N, O, S, que puede estar sustituido por H, OH, F, Cl, Br, I, oxo, alquilo C ₁ -C ₆ , haloalquilo C ₁ -C ₆ , alcoxi C ₁ -C ₆ , haloalcoxi C ₁ -C ₆ , entre otros	 , donde R ¹ : alquil bajo, cicloalquil, fenil	No se menciona
Ar ₂ : fenilo, piridil, tienil, entre otros, sustituido por H, Cl, Br, CN, alquilo C ₁ -C ₆ , haloalquilo C ₁ -C ₆ , alcoxi C ₁ -C ₆ , haloalcoxi C ₁ -C ₆ , entre otros		 , donde R ¹ es H, alquil, halógeno, alcoxi y R: furil, fenil, pirazin, tienil, entre otros
X: O o S		S
R ¹ : H, CN, alquilo C ₁ -C ₆ , haloalquilo C ₁ -C ₆ , alcoxi C ₁ -C ₆ , haloalcoxi C ₁ -C ₆ , entre otros	Q: tienil, tienil sustituido, fenil, fenil sustituido, piridil o piridil sustituido	R ² : H, metil, isopropil y CH ₃
R ² , R ³ y R ⁴ : H, alquilo C ₁ -C ₆ , cicloalquilo C ₃ -C ₆ , alquenoil C ₂ -C ₆ , entre otros que pueden estar sustituidos por F, Cl, Br, I, entre otros, donde R ³ y R ⁴ juntos pueden formar un grupo cíclico saturado o insaturado de 3 a 8 miembros	R ² : H, bajo alquil, bajo alquenoil, piridil, piridil sustituido, entre otros	R ³ que puede ser pirrolidina, piperidina, amino sustituido,  entre otros y

El documento D1 es considerado el estado de la técnica más cercano a la invención definida en la reivindicación 4 porque, divulga compuestos de fórmula (I)


 para el control de insectos y acaros, donde Ar es fenil o piridil sustituido por H, halógeno, haloalquilo, alquil, entre otros; R¹ es alquil bajo, cicloalquil, fenil; Q es tienil, tienil sustituido, fenil, fenil sustituido, piridil o piridil sustituido y R² es H, bajo alquil, bajo alquenoil, piridil, piridil sustituido, entre otros (Pág.1, Párr. [0006] a Pág.2, Párr. [0019]).

La diferencia entre la invención, definida en la reivindicación 1 y el compuesto de D1

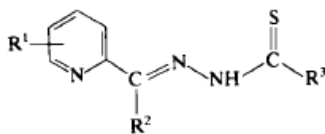
consiste en la sustitución del anillo Ar₂ por una tiosemicarbazona
 
 ,

 mientras que Q esta sustituido por  .

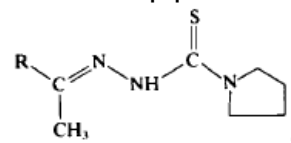
El efecto que lograría las diferencias antes mencionada en el compuesto de fórmula de la reivindicación 1, en cuanto a su acción plaguicida, no puede ser determinado toda vez que no existe alguna evidencia que indique que dichas diferencias estructurales, en cuanto a que la estructura posea una tiosemicarbazona, otorgan al compuesto ahora reivindicado una ventaja técnica sobre los compuestos ya conocidos en la anterioridad D1.

Por lo tanto, el problema técnico objetivo a resolver consiste en plantear una alternativa a los compuestos de fórmula (I) conocidos según D1 para que tenga mejores propiedades plaguicidas.

Sin embargo la estructura de la tiosemicarbazona y su actividad biológica ya es conocida en D2 que divulga la síntesis y los efectos insecticidas de las tiosemicarbazonas, incluyendo las propiedades reguladoras del crecimiento de insectos (Pág. 462). Donde las estructuras de tiosemicarbazonas mencionadas son las



siguientes estructuras generales , donde R¹ es H, alquil, halógeno, alcoxi; R² es H, metil, isopropil y R³ que puede ser pirrolidina, piperidina,



amino sustituido, entre otros (Pág. 463, tabla 1) y la estructura donde R es furil, fenil, pirazin, tienil, entre otros (Pág. 463, tabla 2).

Teniendo en cuenta lo expuesto anteriormente, la personas de nivel medio versada en la materia derivara de manera obvia los compuestos descritos en la reivindicación 1 de la presente invención a partir de las enseñanzas del compuesto del documento D1, y de incluir el hecho que ya es conocida la estructura de la tiosemicarbazona y su actividad biológica mencionado en D2, por lo tanto las diferencias se limitan a variaciones en uno de los sustituyentes, manteniendo los anillos centrales de la estructura, sin que ese hecho confiera una mayor acción plaguicida. Por lo tanto al combinar D1 con D2 la reivindicación resulta obvia.

Puesto que las reivindicaciones dependientes 2 – 7 no mencionan características inventivas adicionales, tampoco pueden ser consideradas inventivas.

Conclusión:

Las reivindicaciones 1 – 7, cumplen el requisito de novedad (Art. 16), pero no cumplen con el requisito de nivel inventivo (Art. 18).

9. Conclusión

Los requisitos de Patentabilidad de la presente Solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	US2002019370	1 – 7	Nivel inventivo
D2	Lars-Erik et Al, Pesticide Science, Dinamarca, vol 25, p 462 - 470	1 – 7	Nivel inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.



MARIA DEL SOCORRO PIMIENTA CORBACHO
Directora de Nuevas Creaciones (E)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2013-07-23

Elaboró: Mayra Alejandra Quintero

Revisó: Lyna Del Carmen Mordecay Dunoyer

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

(Revised manuscript received 6 February 1984)

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.

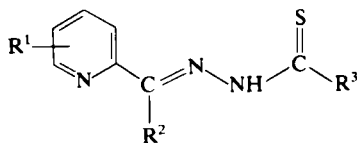
2. Experimental

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



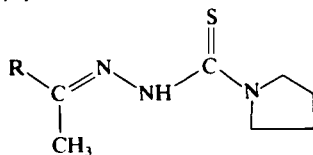
Compound	Structure			M.p. (°C)	Lit. m.p. (°C)	Reference
	R¹	R²	R³			
I	H	Methyl	Pyrrolidin-1-yl	151–152	147–148	14
II	H	H	Pyrrolidin-1-yl	181–184	193–194	17
III	H	Isopropyl	Pyrrolidin-1-yl	157–158.5	161–163	11
IV	5-Ethyl	Methyl	Pyrrolidin-1-yl	72–74		
V	3,5-Dichloro	Methyl	Pyrrolidin-1-yl	116–119		
VI	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		

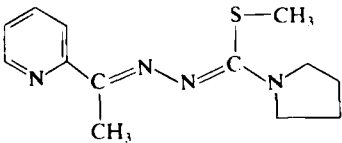
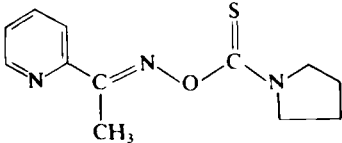
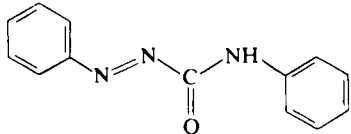
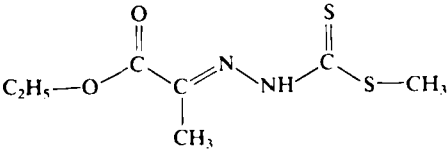
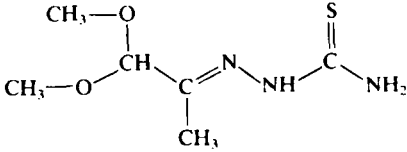
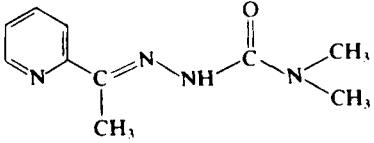
chromatography on a HP 1084 B Liquid Chromatograph by elution with mixtures of a 0.03M phosphate buffer (pH 7) and acetonitrile.

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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