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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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Act. 523 RESPUESTA Folios: 51

Señor Director
DIRECCION DE NUEVAS CREACIONES
Superintendencia de Industria y Comercio
E. S. D.

Trámite : 011
Evento : 379
Actuación : 444

Referencia : Expediente No. **08.082.047**
Asunto : Solicitud de patente para: **SULFOXIMINAS DE (6-HALOALQUILPIRIDIN-3-IL)ALQUILO N-SUSTITUIDAS INSECTICIDAS**
Solicitante : DOW AGROSCIENCES LLC
Fecha solicitud : 06 de agosto de 2008

1. Yo, Luz Clemencia DE PÁEZ, en mi condición de apoderada de la sociedad solicitante de la patente de la referencia, me refiero al oficio No. **9066** notificado por fijación en lista del 01 de junio de 2012.

2. Mediante memorial radicado el día 29 de agosto de 2012 solicité plazo de treinta (30) días para atender el oficio No. **9066**.

3. Mediante oficio No. **9066** se puso en conocimiento del solicitante el informe que contiene las observaciones hechas por su Despacho sobre la presente solicitud de patente. Las observaciones realizadas en el oficio son las siguientes:

3.1 Reivindicaciones relativas a un uso

Señala el Examinador que la reivindicación 9, a pesar de hacer referencia a un método para controlar vegetación insectos, hace referencia al uso de los compuestos reivindicados.

3.2 Reivindicaciones

El Examinador solicita revisar algunos errores de traducción presentes en las reivindicaciones 1 y 9.

De otro lado se considera que la reivindicación 10 no tiene aplicación a nivel industrial.

3.3 Análisis de patentabilidad

3.3.1 Nivel inventivo

Para la evaluación de este requerimiento se cita el documento D1: US 20050228027. Se afirma que el experto en la materia modificaría estructuralmente el radical 6-(C₁-C₄)alquilpiridin-3-il del compuesto revelado en D1, sustituyendo los hidrógenos del alquilo para que contengan halógeno y de esta manera llegar así al objeto de la reivindicación 1 de la presente solicitud.

4. Respuesta a las objeciones

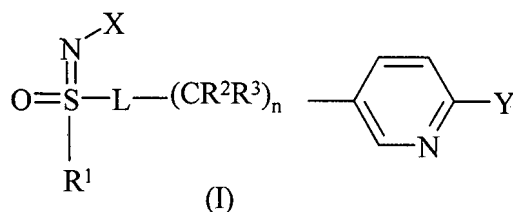
De este modo y con el objeto de atender las objeciones arriba mencionadas, se desea presentar las siguientes modificaciones, aclaraciones y argumentos:

4.1 Modificación de una solicitud en trámite

De acuerdo con el artículo 34 de la Decisión 486 de la Comisión de la Comunidad Andina, donde se establece que la modificación de una solicitud es posible en cualquier momento del trámite mientras no implique una ampliación de la solicitud inicial, me permito presentar un nuevo pliego reivindicatorio conformado por 3 reivindicaciones.

Así, siguiendo las amables sugerencias del Examinador y para presentar la materia de manera más clara, se ha restringido el alcance de la reivindicación 1. La nueva reivindicación 1 recita:

1. *Un compuesto de fórmula (I)*



en donde

X representa CN;

L representa un enlace simple;

*R*1 representa alquilo (C1-C4);

*R*2 y *R*3 representan independientemente hidrógeno o alquilo (C1-C4);

n es un entero de 0 a 3;

Y representa haloalquilo (C1-C4); y

*R*4 representa alquilo (C1-C3).

Esta nueva reivindicación 1 se encuentra debidamente soportada en la descripción y es suficientemente clara para una persona medianamente versada en la materia.

En consecuencia, tanto la descripción como las reivindicaciones y el título presentan la invención de conformidad con las disposiciones actuales y las objeciones formuladas por el Examinador se ven superadas.

4.2 Argumentos a favor del nivel inventivo de la solicitud

El artículo 18 de la Decisión 486 señala lo siguiente:

Artículo 18.- Se considerará que una invención tiene nivel inventivo, si para una persona del oficio normalmente versada en la materia técnica correspondiente, esa invención no hubiese resultado obvia ni se hubiese derivado de manera evidente del estado de la técnica.

Consecuentemente, una invención se considerará inventiva si la misma no hubiese resultado obvia a partir del arte previo, para una persona del oficio normalmente versada en la materia. Esto a su vez implica que el Examinador a cargo de esta evaluación debe colocarse en el momento exacto en el que el inventor buscaba resolver el problema técnico contemplado en la solicitud y para el cual poseía la literatura y el conocimiento disponibles en dicho momento.

Siguiendo entonces los lineamientos de la Jurisprudencia aplicable al análisis del nivel inventivo de una solicitud de patente, se considera que una anterioridad o una combinación de

dos anterioridades puede afectar dicho requerimiento de patentabilidad, si una persona medianamente versada en la materia encuentra enseñanzas o sugerencias claras que, a partir de un conocimiento medio en dicho campo, le permitan llegar de manera obvia a la invención reivindicada. Adicionalmente se considera que una mejora sustancial, un efecto inesperado o una ventaja con respecto al arte previo es un indicio de nivel inventivo, por cuanto aporta un salto tecnológico en la regla técnica.

Respecto a lo anterior, el Tribunal de Justicia de la Comunidad Andina ha expuesto en la Interpretación Prejudicial del proceso 13-IP-2010:

“En este orden de ideas, se puede concluir que una invención goza de nivel inventivo cuando a los ojos de un experto medio en el asunto de que se trate, se necesita algo más que la simple aplicación de los conocimientos técnicos en la materia para llegar a ella, es decir, que de conformidad con el estado de la técnica el invento no es consecuencia clara y directa de dicho estado”.

[señalado fuera del texto]

Por lo anterior, se entiende que para que una persona medianamente versada en la materia pudiera derivar de manera obvia una invención, requeriría simplemente aplicar sus conocimientos técnicos. En contraste, todo aquello que va más allá de la simple aplicación de los conocimientos técnicos y que no ha sido revelado en el estado de la técnica, debe ser considerado inventivo.

Consecuentemente, respetuosamente nos apartamos de la opinión del Examinador en cuanto a la falta de nivel inventivo de la presente solicitud, toda vez que consideramos que los compuestos de la presente solicitud cumplen con dicho requerimiento como se demostrará a continuación:

El documento D1 está relacionado con el campo de los insecticidas, es decir, el control de insectos.

Ahora bien, el análisis llevado a cabo por el examinador no tiene en cuenta que un compuesto, sus propiedades y las interacciones de estas propiedades con un organismo vivo son altamente impredecibles. En el campo de los insecticidas, pueden ocurrir interacciones profundas e impredecibles, aún cuando se cambia incluso un solo átomo. Para demostrar esto se desea poner de presente los siguientes documentos:

1. Kagabu S y S Medej, "Stability Comparison of Imidacloprid and Related Compounds Under Stimulated Sunlight, Hydrolysis Conditions, and to Oxygen" Biosci. Biotech. Biochem., 59 (6), 980-985, (1995):

A. En la Tabla 1, compara el Compuesto 4 y el Compuesto 7 (cambio de un hidrógeno por un grupo metilo) y muestra que la LC_{90} disminuyó en un 80 por ciento.

B. En la Tabla 1, compara el Compuesto 21 y el Compuesto 24 (cambio de un hidrógeno por un grupo metilo) y muestra que la LC_{90} aumentó en 2400 por ciento.

C. En la Tabla 1, compara el Compuesto 25 y el Compuesto 26 (cambio de un hidrógeno por un grupo metilo) y muestra que la LC_{90} disminuyó más del 99 por ciento.

D. En la Tabla 1, compara el Compuesto 4 y el Compuesto 8 (cambio de un hidrógeno por un grupo CF_3) y muestra que la LC_{90} se mantuvo sin cambios.

E. En la Tabla 1, compara el Compuesto 6 y el Compuesto 8, (cambio de un Cl por un grupo CF_3) y muestra que la LC_{90} aumentó en 2400 por ciento.

2. Kagabu S, Murata N, R Hibino, M Hanzawa, y K Nishimura, "Insecticidal and Neuroblocking Activities of Thiamethoxam-Type Compounds in the American Cockroach (*Periplaneta Americana* L.)" Pesticide J. Sci. 30 (2), 111-115 (2005):

A. El artículo afirma que *"Independientemente del anillo heteroaromático, los compuestos NH (15, 17) fueron inactivos en las pruebas insecticidas y de nervios, mientras que los compuestos de metil $[NCH_3]$ 16 y 18 fueron altamente activos en ambas pruebas"* (ver página 114, primera columna).

B. El artículo también afirma que *"incluso un cambio estructural menor, en este caso un sólo átomo de heteroanillo, puede provocar una notable diferencia en el comportamiento biológico ..."* (vea la página 114, segunda columna).

3. Kollmeyer, WD, RF Flattum, JP Foster, JE Powell, ME Schroeder, y SB Soloway, "Discovery of Nitromethylene Heterocycle Insecticides" Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor (páginas 71-89) Eds Yamamoto I, and JE Casida:

A. En la Tabla 1, compara el Compuesto 11 y el Compuesto 12 (cambio de un hidrógeno por un grupo metilo) y muestra que el índice de toxicidad para CEW se incrementó en 2400 por ciento.

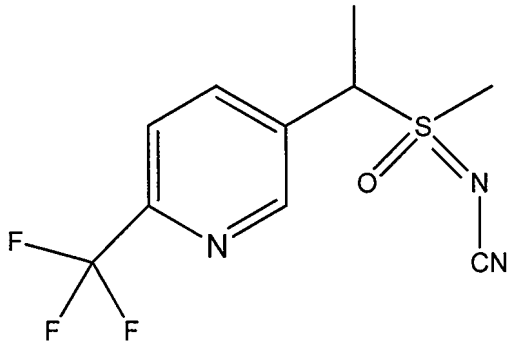
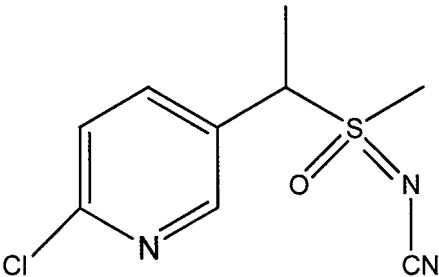
- B. En la Tabla 1, compara el Compuesto 19 y el Compuesto 20 (cambio de un hidrógeno por un grupo metilo) y muestra que el índice de toxicidad para CEW disminuyó alrededor de 92 por ciento.
4. Shiga Y, Okada I, T y Fukuchi, "Synthesis and Acaricidal Activity of N-(1,3,4-Thiadiazol-2-yl)cyclopropanecarboxamides" J. Pesticide Sci. 28, 61-63 (2003):
- A. En la Tabla 1, compara el Compuesto 2 y el Compuesto 1 (cambio de un hidrógeno por un grupo metilo) y muestra que la actividad se incrementó de "totalmente inactivo" a una mortalidad de 100% (Véase el primer párrafo de "Resultados y Discusión").
- B. En la Tabla 3, ambos compuestos 12 y 13 (H vs metilo) "fueron inactivos a 500 ppm" (Véase el tercer párrafo de "Resultados y Discusión").
5. Sparks TC, GD Crouse y D Durst, "Natural Products as insecticides: the biology, biochemistry and quantitative structure-activity relationships of spinosyns and spinosoids" Pest Manag Sci. 57:896-905 (2001):
- A. En la Tabla 1, compara Spinosad B y spinosad A, (cambio de un hidrógeno por un grupo metilo) y muestra que la LC_{50} disminuyó 25 por ciento.
- B. En la Tabla 1, compara Spinosad A y Spinosad D, (cambio de un hidrógeno por un grupo metilo) y muestra que la LC_{50} aumentó 167 por ciento.
6. Wakita T, Kinoshita K, K Kodaka, Yasui N, Naoi A, y Banba S "Synthesis and Structure Activity Relationships of Dinotefuran Derivatives: Modification in the Tetrahydro-3-furymethyl Part" J. Pesticide Sci. 29 (4), 356-363 (2004):
- A. En la Tabla 1, compara el Compuesto 2 y el Compuesto 14 o 16 (cambio de un hidrógeno por un grupo metilo) y muestra que la LC_{70} aumentó aproximadamente 100.000 por ciento.
7. Patente de EE.UU. 4.833.158 "Método para combatir las terminas". Columnas. 9-10:
- A. La Tabla III muestra ciertas ureas de benzoilfenil que son 30 veces menos eficaces en un periodo de prueba de tres semanas, para las cuales el cambio fue sólo un átomo (cambio de F por cualquiera entre H o Cl).

Por consiguiente, es evidente que el cambio de un átomo es impredecible y puede producir resultados notables en las moléculas de insecticidas. En algunos casos, se presenta aumento de la actividad, en otros disminuye, y aún en otros, no hace produce diferencia en absoluto.

El Examinador cita dos referencias bibliográficas y afirma que el flúor mimetiza muy bien el comportamiento de un átomo de hidrógeno. Sin embargo, tal como se demostró anteriormente, el efecto que tiene el cambio de un átomo es impredecible y dependerá de la estructura del compuesto y de sus interacciones internas y externas, por lo que la afirmación del Examinador no puede ser generalizada para todos los casos.

Además, teniendo en cuenta el gran número de posibles sustituyentes y la imprevisibilidad en la técnica insecticida, no es razonable concluir que una persona medianamente versada en la técnica podría seleccionar un sustituyente posible **de los millones existentes** para modificar los compuestos de D1 en una ubicación precisa, **de todas las posibles ubicaciones**.

Por otra parte los siguientes datos muestran claramente la superioridad del compuesto de la reivindicación 2 de la presente solicitud, respecto al compuesto 8 de D1:

Compuesto de la reivindicación 2 ("C2")	Compuesto 8 in D1 ("C8")
	
El valor LC ₉₀ de C2 es 2839% mejor que el valor LC ₉₀ de C8 en contra del pulgón verde del melocotonero	
El valor LC ₉₀ de C2 es 239% mejor que el valor LC ₉₀ de C8 en contra del áfido del algodón	
El valor LC ₅₀ de C2 es 690% mejor que el valor LC ₅₀ de C8 en contra del chinche de planta occidental	

Como se puede observar a partir de los datos anteriores, el compuesto C2 con un haloalquilo, no sólo es mejor que el compuesto C8, con un cloro en lugar de un haloalquilo, sino

que además es sorprendente e inesperadamente mejor. Ninguna persona con experiencia ordinaria en la técnica podría haber predicho tal actividad mayor.

El compuesto de la reivindicación 2 (sulfoxaflor) es el primer producto de la nueva clase de insecticidas de sulfoximina, con un gran espectro de eficacia contra los pulgones, moscas blancas, saltamontes, cochinillas, psílidos y Lygus. Este compuesto actúa sobre los receptores nicotínicos de acetilcolina (nAChR) de los insectos, **de forma distinta respecto a todos los otros insecticidas, incluyendo los neonicotinoides**. Es importante destacar que el sulfoxaflor es eficaz contra cepas de insectos que son resistentes a una amplia gama de insecticidas, incluyendo los neonicotinoides. En particular, en una investigación de terceros, se descubrió que el sulfoxaflor es **28570% mejor** en el control de *Bemisia tabaci* (mosca blanca) multi-resistente que el imidacloprid, el cual es el líder mundial insecticida comercial. Además, en otro estudio, se encontró que el sulfoxaflor es **36467% mejor** en el control de *Nilaparvata lugens* (saltamontes marrón) **resistente al imidacloprid**. La falta de resistencia cruzada al sulfoxaflor en estas cepas resistentes a los insecticidas se correlaciona con la estabilidad metabólica de las monooxigenasas del citocromo P450. La información disponible sugiere que es el mecanismo de resistencia primaria en la mayoría de las especies resistentes a los neonicotinoides. La CYP6G1, una monooxigenasa asociada con la resistencia a neonicotinoides y otros insecticidas metaboliza fácilmente una variedad de neonicotinoides, pero no el sulfoxaflor.

La comunidad internacional ha reconocido la singularidad de la química del sulfoxaflor y su falta de resistencia basada en el metabolismo, de manera que ha ubicado el sulfoxaflor lugar en el subgrupo de modo de acción 4C (Comité de Acción de Resistencia a Insecticidas (IRAC, por sus siglas en inglés), <http://www.irac-online.org/>), diferenciándolo de otros insecticidas del Grupo 4 (4A y 4B).

Por todo lo anterior, los compuestos de la presente solicitud proporcionan un **efecto técnico inesperado** sobre los compuestos del estado del arte, lo cual es un indicio de patentabilidad, ya que las diferencias estructurales de los compuestos reivindicados, así como su efecto técnico asociado no han sido anticipadas por el arte previo.

Así, se puede concluir que los compuestos de la presente solicitud resultan inventivos sobre el estado de la técnica y que la objeción formulada en este sentido se ve superada.

Finalmente, se desea informar que el mérito investigativo aportado por la presente solicitud ha sido reconocido por otras oficinas de patentes del mundo, las cuales han otorgado el privilegio de patente para una invención equivalente a la reivindicada en la presente solicitud,

siendo éste otro indicio de su patentabilidad. La presente solicitud ha obtenido privilegio de patente en los siguientes países o comunidades: Europa, Australia, China, Japón y Estados Unidos.

4.3 Conclusión

Con las modificaciones y argumentos acá presentados, se superan las objeciones formuladas por el Examinador en el oficio No. 9066.

5. Puesto que se ha dado respuesta a todas las objeciones formuladas por el Examinador, se solicita atentamente proceder al otorgamiento de la solicitud de patente para las reivindicaciones 1 a 3.

En este sentido, respetuosamente recordamos al Examinador que la patente no podrá ser negada con base en objeciones diferentes a las expuestas en la acción oficial No. 9066, toda vez que al no haber sido trasladadas a mi representada, dicha decisión violaría tanto el tenor literal del artículo 45 de la Decisión 486 como el derecho constitucional al debido proceso.

En este evento, y con base en el inciso 2 del artículo 45, respetuosamente solicitamos al Examinador expedir una nueva acción oficial que permita a mi representada contra argumentar las mismas o modificar la solicitud con el fin de cumplir los requisitos de patentabilidad.

6. Anexos

- Nuevo pliego reivindicatorio conformado por 3 reivindicaciones
- Formulario de modificaciones en solicitud pendiente
- Kagabu S y S Medej, "Stability Comparison of Imidacloprid and Related Compounds Under Stimulated Sunlight, Hydrolysis Conditions, and to Oxygen" Biosci. Biotech. Biochem., 59 (6), 980-985, (1995).
- Kagabu S, Murata N, R Hibino, M Hanzawa, y K Nishimura, "Insecticidal and Neuroblocking Activities of Thiamethoxam-Type Compounds in the American Cockroach (*Periplaneta Americana L.*)" Pesticide J. Sci. 30 (2), 111-115 (2005).
- Shiga Y, Okada I, T y Fukuchi, "Synthesis and Acaricidal Activity of N-(1,3,4-Thiadiazol-2-yl)cyclopropanecarboxamides" J. Pesticide Sci. 28, 61-63 (2003).
- Sparks TC, GD Crouse y D Durst, "Natural Products as insecticides: the biology, biochemistry and quantitative structure-activity relationships of spinosyns and spinosoids" Pest Manag Sci. 57:896-905 (2001).

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- Wakita T, Kinoshita K, K Kodaka, Yasui N, Naoi A, y Banba S "Synthesis and Structure Activity Relationships of Dinotefuran Derivatives: Modification in the Tetrahydro-3-furylmethyl Part" J. Pesticide Sci. 29 (4), 356-363 (2004).
- Patente de EE.UU. 4.833.158 "Método para combatir las terminas"

Señor Director,


LUZ CLEMENCIA DE PÁEZ

C.C. 35.456.344 de Usaquén

T.P.A. No. 23.555

COLO-10975-0841-020-2
JMS\JMS\ID-227379\WPC10975
No. M-2012-227379\JMS

 Industria y Comercio SUPERINTENDENCIA	Espacio reservado para el adhesivo de radicación
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DIRECCIÓN DE NUEVAS CREACIONES
MODIFICACIONES Y/O CORRECCIONES

1. Identificación del Trámite

☐ Error material

☒ Modificación a una Solicitud en trámite

☒ Patente de Invención

☐ Patente de Modelo de Utilidad

☐ Registro de Diseño Industrial

☐ Esquema de Trazado de Circuitos Integrados

2. Datos del solicitante

Persona Natural

☐

Persona Jurídica

☒

DOW AGROSCIENCES LLC.

Nombre o denominación / Nombre o razón social completa

Nombre representante legal

Tipo de empresa

Micro

☐

Pequeña

☐

Mediana

☐

Otra

☐

Documento de identificación:

☐ C.C.

☐ C.E.

☐ NIT

☐ Otro Número

Nacionalidad/País de constitución	Dirección y domicilio del titular	Ciudad
Estados Unidos de América	9330 Zionsville Road, Indiana 46268	Indianapolis
Dirección electrónica		No. Fax
clientes@cavelier.com		2 11 86 50
		Número telefónico
		3 47 36 11

☐

Autorizo expresamente a la Superintendencia de Industria y Comercio para que todas las comunicaciones sean enviadas a través de la dirección de correo electrónico

3. Datos del apoderado:

Apellidos y Nombre

De Páez Luz Clemencia

Documento de identificación

C.C. 35.456.344

Tarjeta profesional

T.P.A. 23.555

Dirección y domicilio		Ciudad
Carrera 4ª No. 72 –35		Bogotá
Dirección electrónica	No. Fax	Número telefónico
cavelier@cavelier.com	2 11 86 50	3 47 36 11

En caso de haber presentado poder general para asuntos que se adelanten ante la Delegatura de Propiedad Industrial, sírvase indicar el número de su radicación o protocolo de poder general

4. Datos del trámite

Corrección:

Expediente No 08.082.047

Aparece

Debe ser

Modificación:

- ☒ **Modificación al capítulo reivindicatorio.**
Presento nuevo capítulo reivindicatorio compuesto por 3 reivindicaciones y pido respetuosamente que el examen de patentabilidad sea realizado sobre estas nuevas reivindicaciones presentadas.
Respetuosamente manifiesto que a pesar de eliminar materia del pliego reivindicatorio originalmente presentado, mi representada no renuncia a la protección que pueda obtener sobre dicha materia y se reserva el derecho de presentar una solicitud divisional que la contenga durante el trámite de la presente solicitud, beneficiándose de la fecha de prioridad inicialmente reivindicada.
- ☐ **Modificación al capítulo descriptivo.**
- ☐ **Modificación al resumen.**
- ☐ **Modificación a los dibujos.**
- ☐ **Modificación al solicitante.**

5. Anexos

- ☒ Documento que contiene las modificaciones o correcciones.
- ☐ Copia de la solicitud y sus anexos en formato magnético.

6. Firma

Nombre y apellidos

LUZ CLEMENCIA DE PAEZ

C.C. 35.456.344 DE Usaquéen

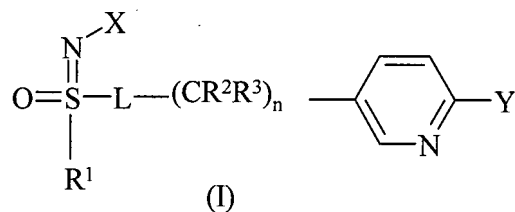
Firma

LUZ CLEMENCIA DE PAEZ

Tarjeta Profesional 23.555

REIVINDICACIONES

1. Un compuesto de fórmula (I)



en donde

X representa CN;

L representa un enlace simple;

R¹ representa alquilo (C₁-C₄);

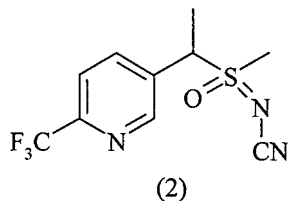
R² y R³ representan independientemente hidrógeno o alquilo (C₁-C₄);

n es un entero de 0 a 3;

Y representa haloalquilo (C₁-C₄); y

R⁴ representa alquilo (C₁-C₃).

2. Un compuesto de acuerdo con la reivindicación 1 en donde dicho compuesto tiene la siguiente estructura



3. Una composición pesticida que comprende una mezcla de:

(a) un compuesto de acuerdo con la reivindicación 2; y

(b) un compuesto seleccionado del grupo que consiste de spinosad, spinetoram, buprofezin, metoxifenoamid, clorpirifos, metil-clorpirifos, gama-cihalotrina, lambda-cihalotrina, pimetozina y piridaben.

Stability Comparison of Imidacloprid and Related Compounds under Simulated Sunlight, Hydrolysis Conditions, and to Oxygen[†]

Shinzo KAGABU and Somporn MEDEJ^{††}

Department of Chemistry, Faculty of Education, Gifu University, Gifu 501-11, Japan

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Lipophilicity and the tendency for decomposition of imidacloprid and related compounds by oxygen, hydrolytic mediums, and simulated sunlight were studied to see whether these physicochemical factors have any relation to the biological activity of the compounds *in vitro*, in a greenhouse, or under field conditions. Lipophilicity indices based on HPLC bore no definite relationship with the binding affinity to the acetylcholine receptor. However, the compounds having high insecticidal potential in greenhouse tests were generally less hydrophilic. In neutral water or in an oxygen-saturated solution, the compounds tested were completely stable. An evident difference was observed in their behavior toward the sunlight wavelength, the nitromethylene compounds decomposing far more rapidly than nitroimines like imidacloprid. The photolability of the nitromethylenes is ascribed to their longer maximum absorption wavelength of over 320 nm.

Imidacloprid (**21**), 1-(6-chloronicotiny)-2-nitroiminoimidazolidine, is a new insecticide with high activity especially against sucking pests.²⁾ The discovery of this compound was the result of seeking improved activity by changing the structure of the originally announced insecticide, 2-nitromethylenetetrahydrothiazine (SKI-71; **33**).³⁾ We have prepared about 2000 compounds and selected imidacloprid for commercial use based on, above all, its insecticidal potential, its lasting effect in greenhouse and field conditions, and its good systemic properties.^{1,2,4-7)} In the study to develop this insecticide, became clear that the 6-chloro-3-pyridylmethyl group was an essential structural element for insecticidal activity, and we have called imidacloprid and its related insecticide class "chloronicotinoids" after the common name of the 3-pyridylmethyl group.⁷⁾

Electrophysiological studies of the chloronicotinoids⁸⁾ and their inhibition of α -bungarotoxin binding⁹⁾ indicated that they had the same mode of action as the natural insecticide, nicotine, and the binding affinity of a series of compounds in the insect receptor has been evaluated.⁸⁻¹⁰⁾ However, the listed intrinsic activity expressed by binding affinity or *K_i* values, the minimum concentration in μ M to bind to the honeybee head nicotinic acetylcholine receptor, did not always match the actual insecticidal activity, LC₉₀, the concentration in ppm to kill 90% of the green rice leafhopper in greenhouse tests (Table I). A pertinent example is the most potential insecticide in this class, imidacloprid (**21**), which showed inferior activity in this *in vitro* system to nitromethylene analogs **6**, **12**, **15**, and **17**. The discrepancy between the binding affinity and the insecticidal activity in the greenhouse or field suggested the involvement of factors other than the affinity of the drug to the receptor.

We have focussed in this report on some physicochemical properties associated with the fate of the compounds under environmental conditions; that is, their stability toward hydrolytic mediums, oxygen, and sunlight. In addition, we have examined the lipophilicity of these compounds, a factor

especially connected with the *in vivo* behavior of the molecules. However, we have not dealt here with the behavior in soil, another important degradation pathway for pesticides.

Materials and Methods

Materials. The reagents and solvents used were of analytical grade. The following compounds were prepared according to the descriptions in the literature: **1** and **2**¹¹⁾; **3**, **4**, and **5**¹²⁾; **6**⁴⁾; **7**, **8**, **9**, **10**, and **11**¹³⁾; **12**⁶⁾; **13**⁴⁾; **14**¹⁵⁾; **15**¹⁶⁾; **16**¹⁷⁾; **17**³⁾; **18**¹⁷⁾; **20**¹⁸⁾; **21**¹⁾; **23**¹⁹⁾; **25**, **26**, and **27**²⁰⁾; **28** and **29**²¹⁾; **30** and **31**²²⁾; **32**²³⁾ and **33**.²⁴⁾

Preparation of 19. To a solution of *N*-(6-chloro-3-pyridyl)methylethylenediamine (1.86 g, 10 mmol) and triethylamine (2.02 g, 20 mmol) in acetonitrile (50 ml) was added bis(trichloromethyl)carbonate, "triphosgene" (2.97 g, 10 mmol), in one portion, the mixture being stirred at room temperature for 3 h and then heated under reflux for another 3 h. The reaction mixture was poured into water and basified with 5% sodium hydroxide to destroy the excess triphosgene. The organic phase was extracted with chloroform, washed with water, and dried. The solvent was evaporated, and the residue was chromatographed on silica gel by eluting with chloroform-ethanol (10:1, v/v). The crude product was recrystallized from ethanol; yield 630 mg (29%); mp 144°C; IR ν_{max} (KBr) cm^{-1} : 3240, 1690; NMR δ_{H} (90 MHz, DMSO-*d*₆): 3.22 (4H, br. s), 4.25 (2H, s), 6.45 (2H, s), 7.46 (1H, d, *J*=8.1 Hz), 7.70 (1H, dd, *J*=8.1 and 2.4 Hz), 8.27 (1H, d, *J*=2.4 Hz). *Anal.* Found: C, 51.07; H, 4.76; N, 19.85%. Calcd. for C₉H₁₀ClN₃O: C, 51.07; H, 4.76; N, 19.85%.

Preparation of 22. A solution of *N*-(6-chloro-3-pyridyl)methylethylenediamine (1.86 g, 10 mmol) and α -cyanoacetimidate (1.12 g, 10 mmol) in acetonitrile (50 ml) was heated under reflux for 3 h. The solvent was distilled off, and the residue was chromatographed on silica gel, eluting with chloroform-ethanol (10:1, v/v). The crude product was recrystallized from ethanol; yield 920 mg (39%); mp 128°C; IR ν_{max} (KBr) cm^{-1} : 3280, 2200, 1500; NMR δ_{H} (90 MHz, CDCl₃): 3.27-3.67 (4H, m), 4.18 (2H, s), 4.83 (1H, br. s), 7.30 (1H, d, *J*=7.5 Hz), 7.55 (1H, dd, *J*=7.5 and 2.4 Hz), 8.25 (1H, d, *J*=2.4 Hz). *Anal.* Found: C, 56.12; H, 4.73; N, 23.96%. Calcd. for C₁₁H₁₁ClN₄: C, 56.29; H, 4.72; N, 23.88%.

Preparation of 24. Imidacloprid (256 mg, 1 mmol) was added in portions over a period of 1 h to a suspension of sodium hydride (24 mg, 1 mmol, 60% oil dispersion) in 5 ml of DMF at 0°C under a nitrogen atmosphere. After dimethyl sulfate (124 mg, 1.2 mmol) had been added dropwise, stirring was continued for 10 h. The reaction mixture was poured into 30 ml of cold water, and the precipitated solid was filtered and washed thoroughly

[†] Chloronicotinyl Insecticides, Part VI. Part V, see ref. 1; Part VII was published, see ref. 32.

^{††} A fellow of the Certification in Service Training Program for Foreign Teachers (Gifu University, Oct. 1992-March 1994).

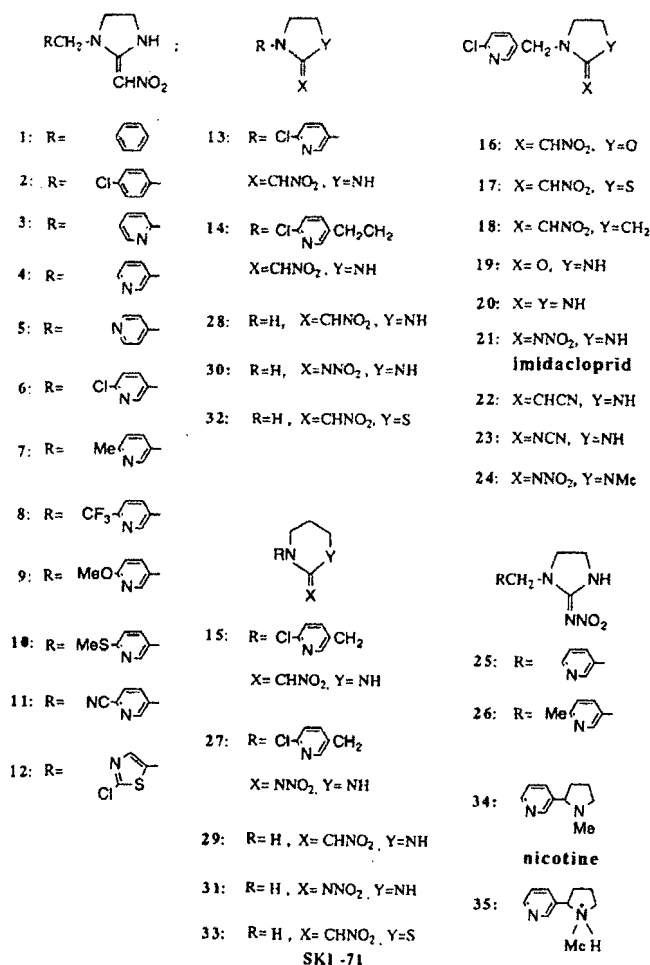


Fig. Imidacloprid and Related Compounds.

with water. The air-dried precipitate was washed with hexane and subjected to preparative thin-layer chromatography on silica gel with a 95:5 (v/v) mixture of dichloromethane and ethanol as the eluting solvent. The band of R_f 0.3–0.5 was extracted with chloroform, and the crude product was recrystallized from ethanol, affording 27 mg (10% yield) as colorless needles of mp 108°C; IR $\nu_{\max}$ (KBr) cm^{-1} 1590; NMR δ_H (90 MHz, CDCl_3): 2.98 (3H, s), 3.38–3.90 (4H, m), 4.48 (2H, s), 7.34 (1H, d, $J=7.8$ Hz), 7.68 (1H, dd, $J=7.8$ and 2.4 Hz), 8.28 (1H, d, $J=2.4$ Hz). Anal. Found: C, 44.22; H, 4.46; N, 25.72%. Calcd. for $\text{C}_{10}\text{H}_{12}\text{ClN}_3\text{O}_5$: C, 44.54; H, 4.48; N, 25.97%.

HPLC measurement. The HPLC equipment consisted of a pump (BIP-I, JASCO) and a UV detector (UVIDEC 100-VI, JASCO) with a recorder (CR6A CROMATOPAC, Shimadzu). A wavelength of 254 nm was used to detect the test compounds. The sample (10 μl of about 20.0 $\mu\text{g}/\text{ml}$) was introduced by using an injection syringe with a 50 μl needle (MICROLITER, Hamilton) and separated in 5- μm Lichrosorb RP-18 columns (4.0 ϕ $\times$ 250 mm; Merck) or ODS L-Columns (4.6 ϕ $\times$ 150 mm; Waters Associates). For the mobile phase, acetonitrile, methanol, and deionized water were freshly distilled before use. The buffer solutions were prepared according to the standard descriptions,²³ and the pH values were checked by a pH meter (F-11 pH-Meter, Horiba).

The capacity factor, k , of the solutes is expressed in terms of the retention times of the sample compound (t) and of the reference compound, potassium bromide or potassium nitrate (t_0), according to Eq. 1.²⁶

$$k = (t_r - t_0)/t_0 \quad (1)$$

The presented log k values are the mean values of three runs under the given conditions.

The UV spectra were obtained with a UV-160A instrument (Shimadzu) in the given solvents.

Photodegradation experiment. A solution of 20 μg of the test compound in 20 ml of a mixed solvent of acetonitrile and water (8:2, v/v) was prepared in a 50-ml round-bottomed Pyrex flask. The flask was immersed to one-third height in a thermostatic bath maintaining the water temperature at 30°C and rotated. Irradiation was provided by a 250 W sun lamp (NC 7222 Sun Light Beam Lamp, National) maintaining a light intensity of 103,200 lux (checked by a Photocell Illuminator, Toshiba). A 0.5 ml aliquot was taken every 30 min and measured by HPLC with elution by acetonitrile and water (8:2, v/v) in a 5- μm Lichrosorb RP-18 column (4.0 ϕ $\times$ 250 mm; Merck).

Stability experiment in an oxygen-saturated solution. A 500 ml aqueous solution of the compound at a concentration of 10 ppm was prepared in a 1-liter round-bottomed Walter-type bottle. While maintaining the inner temperature at 30°C in a thermostatic bath, oxygen gas was allowed to flow into the solution through the spiral tube at a flow rate of 20 cm^3/min . Ten microliters of the sample solution was taken at the given times and analyzed by HPLC.

Decomposition in neutral, acidic, and basic solution. A solution of the test compound was left to stand in a stop-cocked flask at 26°C, or was stirred with a condenser attached to the flask at elevated temperatures in the dark, an aliquot being taken at the given times and analyzed by HPLC.

Partition coefficient of imidacloprid (21) between octanol and water. A sealed ampule containing 100 mg of 21, 100 ml of water and 100 ml of octanol was continuously shaken for 48 h at 22°C. After standing the mixture for 10 h, each phase had separated and the solvent was evaporated to dryness. The phases were finally dried under vacuum until the weight did not decrease any more. The average value for log $P_{\text{octanol/water}}$ (log $P_{O/C}$) was 0.589 from three runs.

Results

HPLC measurement of compounds 1–33 under neutral conditions (Table I)

We can summarize the listed $\log k$ values as follows: ① Introduction of a benzyl group to the 1-*N* atom of 2-nitromethyleneimidazolidine increased $\log k$ (0.497 for 1 vs. –0.230 for 28), but the enhancement was not much in the case of a pyridylmethyl group (3, 4, and 5 vs. 28). ② The $\log k$ value increase was magnified by the substituent on the aromatic ring such as Cl, Me, CF₃, MeS, (2 vs. 1; 6, 7, 8, and 10 vs. 4), and this enhancing effect by the substituted aralkyl groups was also found in the nitroimino series (e.g. 0.346 for 26 vs. –0.150 for 30) and the hexahydropyrimidine homolog (15 vs. 29; 27 vs. 31). ③ The $\log k$ values among the saturated heterocyclic rings could be ranked six-membered > five-membered ring (29 > 28, 31 > 30, 33 > 32) on one hand, and for the ring atoms S > C > O > N (17 > 18 > 16 > 13) on the other hand. ④ The nitroimines had higher values than the nitromethylenes (30 > 28, 31 > 29, 21 > 6, 27 > 15).

HPLC measurements of compounds 1, 4, 6, and 21 and nicotine under acidic, neutral, and basic conditions (Table II)

The $\log k$ values for the imidazolidines with a nitromethylene or nitroimino group at the 2-position were almost unchanged in the pH range from 5.80 to 9.88 (1, 4, 6, and 21), while the values for nicotine in the acidic and neutral ranges, 0.053 and 0.076, respectively, increased greatly to 0.587 at pH 9.88.

Stability of compounds 6, 17, 21, and 25, and nicotine in an oxygen-saturated solution (Table III)

Nitromethylenes 6 and 17, and nitroimines 21 and 25 were quite stable under the given conditions, while some degradation of nicotine was recognized.

Stability of compounds 6, 21, 23, and 32 under hydrolytic conditions (Table IV)

Tested nitromethylenes 6 and 32, nitroimine 21, and cyanoimine 23 were generally stable in both acidic and neutral conditions at ambient temperature, remaining practically unchanged during 10–24 weeks. At 60°C, we recognized the decomposition of 21 by 26% of its initial content after 24 h. On the other hand, nitroimine 21 was rather unstable under basic conditions; it was decomposed by 21% at pH 9 and 26°C in 24 h. In comparison, the nitromethylenes were apparently more stable; hydrolysis took place at higher temperatures, being 20% for 6 and 40% for 32 at pH 9 and 60°C in 24 h, and 40% for 33 at pH 10.3 and 85°C in 10 h. Cyanoimine 23 was also stable in an alkaline solution.

Degradation by simulated sunlight (Table V)

The photolysis experiment was carried out in a mixed solvent of acetonitrile and water, because of the insufficient solubility of some compounds in water alone. A notable general phenomenon for photolysis was that the nitromethylene derivatives decomposed more rapidly than the nitroimine and cyanoimine partners. A pertinent example is that chloronicotiny-2-nitromethyleneimidazolidine (6) decomposed by 57% with 1.5 h of irradiation, while the half-life period for imidacloprid (21) was 3.0 h and cyanoimine (23) decayed by only 2% in 24 h. Oxazolidine (16), thiazolidine (17), and pyrrolidine (18), which bear a nitromethylene group, decomposed mostly within an hour. On the other hand, *N*-methylation of imidacloprid (21) affected the photostability only a little (60% degradation of 21 vs. 52% of 24 after 4 h). Compound 27, the six-membered homolog of imidacloprid, appeared to be a little more stable than 21. SKI-71 (33), lost 70% of its initial content within one hour.

Table I. $\log k$ Values for Compounds 1–33 in Neutral Conditions,^a Together with the Insecticidal Activity (LC₉₀)^b and Binding Affinity to the Acetylcholine Receptor (K_i)^c

Compd.	$\log k$	LC ₉₀	K _i	Compd.	$\log k$	LC ₉₀ ^b	K _i ^c	Compd.	$\log k$	LC ₉₀ ^b	K _i ^c
1	0.497	200	2.46	13	0.211	8	92.1	24	0.314	8	164
2	0.833	40	1.17	14	0.231	40	11.1	25	0.138	1000	7.29
3	–0.081	200	12.1	15	0.173	0.32	0.028	26	0.346	1.6	
4	–0.130	8	0.083	16	0.244	8	0.308	27	0.382	8	
5	–0.112	8	136	17	0.641	0.32	0.116	28	–0.230	1000	
6	0.204	0.32	0.021	18	0.622	1.6	0.359	29	–0.150	1000	
7	0.122	1.6	0.473	19	0.230	1000		30	–0.150	1000	
8	0.488	8		20		1000	3.08	31	–0.110	1000	
9	0.174	40		21	0.504	0.32	1.55	32	0.073	1000	
10	0.323	1000			(0.589) ^d			33	0.075	40	1082
11	0.088	200		22	0.250	—			(0.4) ^e		
12	0.214	0.32	0.081	23	0.477	1.6	6.88	nicotine		1000	1.68

^a Column. 4.0φ × 250 mm, mobile phase, acetonitrile/water (3:2, v/v); flow rate, 0.3 ml/min; column temperature, 25.0 ± 0.5°C; reference substance, KNO₃.

^b Taken from refs. 1, 2, 4, 5, 6 and 7; LC₉₀ is the concentration in ppm to kill 90% of the green rice leafhopper tested by using pots in a laboratory (— represents no activity at a 1000 ppm dose).

^c Taken from ref. 9; K_i is the minimum concentration in μM to bind to the honeybee head nicotinic acetylcholine receptor.

^d Log *P*_{01c} value for 21, this work.

^e Log *P*_{01w} value for 31 taken from ref. 3.

Electronic absorption spectra in water (Table VI)

The longest-wavelength band of 2-nitromethyleneimidazolidine (28) was 312.5 nm, which was shifted bathochromically to around 320 nm by the introduction of an unsubstituted or substituted aralkyl group to the 1-nitrogen atom of the imidazolidine skeleton (see compounds 1–14). For larger bathochromic shifts were observed in the other heterocyclic rings than with the aforementioned nitromethyleneimidazolidines; for thiazolidines 32 and 17 at 346 nm and 357 nm, respectively, and for oxazolidine 16 and pyrrolidine 18 at 335.5 nm and 354.5 nm, respectively. Six-membered tetrahydrothiazine 33 had its maximum absorption at a longer wavelength of 348 nm. In contrast, the imidazolidines containing a nitroimino moiety like 30, 21, and 27 absorbed a far shorter wavelength of 265–269 nm than the nitromethylene analogs. Further, a hypsochromic shift from 28 by as much as 45 nm was observed in cyanoimine 23.

Discussion

Insecticidal activity is an integrated effect comprising intrinsic activity at the site of action, *in vivo* behavior like excretion or penetration, and stability on the host plants in the open as well as in soil. Lipophilicity is a factor to influence on the fate of molecules on the route from penetration into insect cuticle until the interaction with the receptor occurs. The relationship between the molecular

Table II. Log *k* Values for Compounds 1, 4, 6, and 21 and Nicotine under Neutral, Acidic and Basic Conditions^a

Compd.	log <i>k</i>		
	5.80 ^b	pH of mobile phase 7.10 ^c	9.88 ^d
1	0.337	0.341	0.324
4	0.075	0.076	0.074
6	0.178	0.180	0.173
21	0.290	0.293	0.279
Nicotine	0.053	0.076	0.587

^a Column, ODS L-column (4.6φ × 150 mm); flow rate, 0.7 ml/min; column temperature, 21.0 ± 0.5°C; reference substance, KBr.

^b Prepared by mixing methanol with an equi-volume of 20 mM aqueous NaH₂PO₄ · 2H₂O.

^c Mobile phase: methanol/water (1:1, v/v).

^d Prepared by mixing methanol with an equi-volume of 20 mM aqueous Na₂HPO₄ · 12H₂O.

Table III. Degradation in an Oxygen-saturated Solution

Compd.	Content (%) ^a						
	Time (h)						
	1.0	2.0	4.0	8.0	16.0	24.0	48.0
6	100	100	100	100	100	100	100
17	100	100	100	98	95	94	93
21	100	100	100	100	100	100	100
25	100	100	98	95	96	94	90
Nicotine	99	98	97	95	90	85	80

^a Percentage of the peak area of the compound based on 100% at the initial time by HPLC.

Table IV. Decomposition of Compounds 6, 21, 23, and 32 under Hydrolytic Conditions

Compd.	Temp. (°C)/pH ^b	Content (%) ^a									
		Time (wk or h) ^c									
		1	2	3	4	5	6	8	10	24	
6	26/ 4.7	100	99	100	100	100	100	100	100		
	26/ 7.7	100	99	100	95	97	100	100	100		
	26/ 9.0	100	97	100	98	95	94	94	90		
	60/ 4.7	100	100	97	100	100	100				
	60/ 7.7	100	100	100	100	100	100	100	100		
	60/ 9.0	99	100	99	90	88	85			80	
	80/10.3	93	96	95	90	85	80			73	
	26/ 4.7	100	96	100	97	98	98			98	
21	26/ 7.7	100	100	100	100	97	98			97	
	26/ 9.0	100	93	90	87	84	82	80	80	79	
	60/ 4.7	100	100	100	100	100	100			100	
	60/ 7.7	98	93	90	85	81	78			76	
	60/ 9.0	58	35	19	12	7	5			0	
	85/10.3	50	30	16	9	5	2			0	
	26/ 4.7	100	99	100	99	96	96		96		
	26/ 7.7	100	100	100	97	97	98		98		
23	26/ 9.0	100	100	100	100	100	100		100		
	60/ 4.7	100	100	99	100	99	100		100		
	60/ 7.7	100	100	100	100	99	100		100		
	60/ 9.0	99	100	99	100	99	100			95	
	85/10.3	100	100	100	95	93	91			88	
	26/ 4.7	100	100	100	100	100	98	97	98		
	26/ 7.7	100	100	100	100	100	100	94	96		
	26/ 9.0	100	100	99	99	95	96	94	89		
32	60/ 4.7	100	100	100	100	98	100				
	60/ 7.7	100	100	100	100	100	100	95	90		
	60/ 9.0	80	80	77	75	73	70			60	
	85/10.3	97	87	80	77	72	69		60		
33											

^a Percentage of the peak area of the compound based on 100% at the initial time by HPLC.

^b The solutions corresponding to pH 4.7, 7.7, 9.0, and 10.3 were respectively 1) a mixture of 6.95 ml of 2 M NaOAc, 3.05 ml of 2 M HOAc and 100 ml of water, 2) water, 3) 20.8 ml of 0.1 M NaOH, 50 ml of an aqueous solution containing 3.092 g of H₃BO₃ and 3.728 g of KCl, and 4) an equi-volume mixture of the aforementioned buffer for pH 9.0 and ethanol.

^c No. of weeks for the runs at 26°C; hours for the other runs at the elevated temperatures.

Table V. Degradation by Simulated Sunlight

Compd.	Content (%) ^a										
	Time (h)										
	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	24.0
4	75	59	54	47							
6	85	67	43	20	13						
7	91	71	54	40							
15	90	78	66	57	47	41					
16	33	10									
17	28										
18	15										
21	93	83	73	65	54	49	45	40	35	30	
23	100	99	99	99	100	99	100	99	100	100	98
24	90	82	75	70	64	59	54	48			
27	95	88	79	70	63	59	53	49	45	40	
33	66	30									

^a Percentage of the peak area of the compound based on 100% at the initial time by HPLC.

Table VI. Electronic Absorption Spectra in Water

Compd.	$\lambda_{\text{max}}(\text{nm})/\log \epsilon$
1	318.5/4.20, 223.0/3.49
2	320.0/4.14, 219.4/3.90
3	319.8/4.28, 274.0/3.70, 265.0/3.69, 229.0/3.71
4	321.0/4.32, 268.0/3.63, 262.0/3.61, 223.0/3.68
5	320.0/4.38, 253.0/3.63, 247.0/3.60, 211.0/3.70
6	323.0/4.09, 265.0/3.56, 214.0/3.81
7	322.5/4.18, 271.0/3.65, 265.0/3.63, 205.0/3.82
8	322.9/4.23, 265.0/3.51, 259.0/3.49
9	321.5/4.08, 286.0/3.71, 218.0/3.85
10	319.0/4.36, 243.0/4.06
11	324.0/4.09, 265.0/3.34, 221.0/3.99
12	324.8/4.17, 242.0/3.66
13	329.3/4.35, 277.0/3.37, 268.0/3.42
14	323.5/3.98, 277.0/3.56, 213.9/3.76
15	313.0/4.17, 277.0/3.90, 212.8/3.95
16	335.5/4.12, 266.8/3.65, 213.0/4.16
17	357.0/4.23, 277.0/3.37, 268.0/3.42, 211.2/3.92
18	354.5/4.54, 277.0/3.64, 268.0/3.75, 213.4/4.20
19	269.0/3.56, 212.8/4.03
20	269.0/3.32, 213.0/3.89
21	269.0/4.17, 211.0/3.96
22	268.5/3.69, 212.5/4.07
23	267.8/3.56, 214.8/4.35
24	255.4/4.10, 213.0/4.19
25	268.5/4.20
26	267.5/3.64, 207.0/3.41
27	269.0/3.61, 213.0/4.05
28	312.5/4.24, 224.5/3.63
29	334.0/2.76, 301.0/3.63, 220.0/3.11
30	265.5/3.85, 208.0/3.28
31	265.0/4.01, 213.0/3.59
32	346.0/4.59, 230.0/3.49, 202.0/3.56
33	348.0/4.07, 290.0/3.29, 230.0/3.29, 208.0/3.31

lipophilicity and the retention index by HPLC, $\log k$, has long been studied, and quantitative relationships have successfully been applied to biological molecules.^{27,28} Table I lists $\log k$, the *in vivo* insecticidal potential (LC_{90}), and the *in vitro* binding affinity (K_i). However, no systematic relationship between the $\log k$ and K_i values is apparent. An illustrative example is the pyridylmethyl isomers (3, 4, and 5), which are obviously different in their binding affinity despite having the same order of lipophilicity. On the other hand, as for the relationship between $\log k$ and LC_{90} , the compounds having higher LC_{90} values are generally less hydrophilic, as can be seen with 6, 7, 12, 15, 17, 18, 21, 23, and 26. Another observation is that the nitroimine compounds were more lipophilic than their nitromethylene partners, as exemplified by 21 against 6, although 21 itself is taken as rather hydrophilic, judging from its lower $\log P_{\text{o/w}}$ 0.589 under the general criterion of 0.5–3.5 for systemic insecticides.

Nicotine 34 is far weaker in insecticidal activity than imidacloprid 21 (LC_{90} of 1000 vs. 0.32), although both have the nearly the same level of binding affinity to the receptor. Yamamoto and Tomizawa have attributed the inferior insecticidal activity of nicotine to its basicity.⁹ Since 34 exists overwhelmingly in the ionized form of 35 in the neutral pH range due to the basic nitrogen atom on the pyrrolidine ring, its penetration into the central nervous system through lipophilic fluids is greatly restricted. On the other hand,

chloronicotinoids like 21, as the corresponding nitrogen atom on the imidazolidine ring partakes rather positive by the contiguous strong electron-withdrawing group, can go through ionic barriers easily. Table II shows this tendency: the closeness of $\log k$ values for nicotine in the pH 7.10–5.80 range means that molecules are already protonated in the neutral pH region, and the remarkably lengthened retention at pH 9.88 indicates in turn that they are deprotonated in this basic region and behave as free bases. In contrast, the retention value for 21 remains almost unchanged in a wide pH range.

Table III shows that the selected pyridylmethyl compounds were wholly stable toward oxygen.

The tested chloronicotinoids (6, 21, and 23) and thiazolidine 32 were, as a whole, quite stable in an acidic solution and were stable in a neutral solution (Table IV). In a basic medium the stability of 21 was appreciably inferior to that of nitromethylenes 6 and 32.

The photostability study in Table V presents definitive evidence for the superiority of 21 in field application to the nitromethylene analogs like 6, the half-life period of 21 under simulated sunlight irradiation being over twice longer than that of 6. Our lead compound 33, judging from its photolability, would lose its insecticidal activity rapidly when exposed in the field.

The photostability of a molecule can be judged on the basis of its electronic absorption spectra (see, Table VI). Sunlight has a wavelength of more than 290 nm on the earth.²⁹ It is self-evident that nitromethylenes with the chromophores of 320–360 nm are liable to lose its insecticidal activity in the field as a result of molecular excitation and subsequent structural degradation. The maximum absorption of 33 has been assigned to the π -electron transition from the enamine portion of the molecule to the nitro group.³⁰ The shorter wavelength of nitroimines and cyanoimines than of nitromethylenes is rationally ascribable to the stronger π -bond strength for $\text{CH}_2=\text{NH}$ than $\text{CH}_2=\text{CH}_2$ (74.2 vs. 63.1 kcal/mol).³¹ The hypsochromic shift (13.6 nm) of 24 by methylation of the nitrogen atom of the imidazolidine ring of 21 is probably due to the perturbed mesomery resulting from the distorted planarity of guanidyl part by steric congestion. It is puzzling why the photostability of 24 was nevertheless improved only modestly. Some photolytic processes independent of π -electron transition such as demethylation may occur.

Conclusions: The binding affinity of imidacloprid was inferior to that of nitromethylene analogues. Furthermore, it tended to decompose more readily than its competitors under hydrolytic conditions. Notwithstanding, imidacloprid has been selected for commercial use. This study shows clearly that its photostability is a dominant factor for its application.

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Note

Insecticidal and Neuroblocking Activities of Thiamethoxam-Type Compounds in the American Cockroach (*Periplaneta americana* L.)

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Insecticidal thiamethoxam and related heterocyclic compounds were examined using American cockroaches to see if they decayed to the acyclic neonicotinoid clothianidin and what was responsible for activity. The minimum insecticidal dose was 1.4 nmol for thiamethoxam, and 2.0 mmol for clothianidin, while the doses for triazine and thiadiazine analogues were evidently larger than that for clothianidin. In a physiological salt solution of thiamethoxam for 7 days, the appearance of clothianidin could not be confirmed, while the related compound triazine partly decayed to clothianidin. From these results, the prodrug concept does not necessarily apply to thiamethoxam but does to the triazine in the present experiment. Insecticidal and neuroblocking tests were also conducted for three chloronicotinyl derivatives of thiamethoxam, triazine and thiadiazine analogues, six *N*-desmethyl derivatives and three acyclic clothianidin analogues. © Pesticide Science Society of Japan

Keywords: prodrug, thiamethoxam, clothianidin, insecticidal potency, American cockroach.

INTRODUCTION

Successful designs of a molecule which has to be transformed in order to show or enhance the biological activity of the original structure, a so called prodrug, are found often in developed pesticides.^{1,2)}

The neonicotinoid thiamethoxam (2) may be taken as one example. This commercial insecticide shows highly effective field action especially in the control of chewing and sucking insects, common targets for imidacloprid (14), clothianidin (16) and other

neonicotinoids. However it shows far less binding affinity than other neonicotinoids in housefly head membrane preparations and also in electrophysiological whole cell voltage clamp studies neurons isolated from *Heliothis virescens* ventral nerve cord showed no response to thiamethoxam when applied at a concentration of 0.3 mM.^{3,4)} The oxadiazine framework of thiamethoxam is constructed by the dehydrative condensation of acyclic diamine, formaldehyde and formic acid, and hence the hydrolytic regeneration of the starting components is conceivable.

Judging from its biological features and structural makeup, the diamine component of clothianidin (16), another neonicotinoid insecticide,⁵⁾ is suspected to be responsible for the actual insecticidal activity of thiamethoxam. In fact Nauen *et al.* revealed by LC-MS/MS analysis that thiamethoxam was rapidly metabolized to clothianidin when orally administered to *Spodoptera frugiperda* larvae or applied to cotton plants.⁶⁾

On the other hand, Wiesner and Kaiser³⁾ and Maienfisch *et al.*⁴⁾ concluded that the activity of thiamethoxam was due to itself with its unique mode of binding to the target sites distinct from that of other neonicotinoids in light of a binding experiment using aphid membranes.

We have been studying the biological activity of neonicotinoid insecticides through injection and neurophysiological measurements using the American cockroach.^{7–10)} We examined thiamethoxam in the present study. Our study also includes triazine and thiadiazine analogues (5–12).^{11–15)} The prodrug concept can apply to these compounds because they are constructed in a similar fashion as thiamethoxam, *i.e.* condensation of clothianidin (or its chloropyridyl derivative) with formaldehyde and individual heteroatom sources.

MATERIALS AND METHODS

1. Materials

Imidacloprid (14)/clothianidin (16) and compounds 7/8 were obtained from Bayer CropScience Co. Ltd. and AgroKanesho Co. Ltd., respectively. The methods used to prepare compounds 1–12,^{16,17)} 13¹⁸⁾ and 15–18^{16,17)} have been described together with some physical data. Here only NMR spectral data not available in the literature for compounds 6, 8–10, 12 and 13 were provided with chemical shifts in δ (ppm) and the coupling constants in Hz.

6: ¹H-NMR (CDCl₃, δ) 2.50 (3H, s), 3.06 (3H, s), 4.32 (2H, s), 4.33 (2H, s), 4.76 (2H, s), 7.50 (1H, s); ¹³C-NMR (CDCl₃, δ) 35.8, 39.9, 44.3, 67.2, 70.4, 134.2, 141.1, 154.1, 156.9. 8: ¹H-NMR (CDCl₃, δ) 2.49 (3H, s), 3.09 (3H, s), 4.24 (2H, s), 4.33 (2H, s), 4.68 (2H, s), 7.36 (1H, d, *J*=8.1), 7.80 (1H, dd, *J*=8.1/2.6), 8.82 (1H, d, *J*=2.6); ¹³C-NMR (CDCl₃, δ) 35.6, 39.9, 48.7, 67.1, 70.4, 124.9, 129.5, 139.4, 149.2, 151.9, 157.4. 9: ¹H-NMR (CD₃COCD₃, δ) 4.57 (2H, s), 4.73 (2H, s), 4.81 (2H, s), 7.71 (1H, s), 10.14 (1H, bs); ¹³C-NMR (CD₃COCD₃, δ) 43.6, 47.9, 49.5, 136.7, 12.4, 153.0, 155.6. 10: ¹H-NMR (CDCl₃, δ) 3.23 (3H, s), 4.44 (2H, s), 4.48 (2H, s), 4.86 (2H, s), 7.52 (1H, s);

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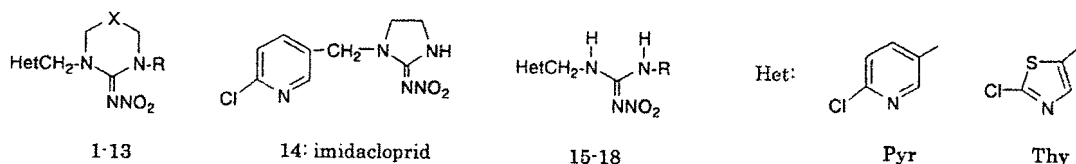


Fig. 1. Test compounds and imidacloprid.

¹³C-NMR (CDCl₃, δ) 40.1, 48.0, 48.7, 51.4, 133.6, 134.9, 141.4, 157.8. 12: ¹H-NMR (CDCl₃, δ) 3.28 (3H, s), 4.36 (2H, s), 4.51 (2H, s), 4.83 (2H, s), 7.37 (1H, d, *J*=8.5), 7.81 (1H, dd, *J*=8.5/2.0), 8.34 (1H, d, *J*=2.0); ¹³C-NMR (CDCl₃, δ) 40.2, 48.2, 51.4, 53.0, 108.7, 125.1, 139.5, 156.4, 164.8, 167.4. 13: ¹H-NMR (CDCl₃, δ) 2.03 (2H, m), 3.37 (2H, m), 3.50 (2H, m), 4.71 (2H, s), 7.33 (1H, d, *J*=8.6), 7.76 (1H, dd, *J*=8.6/2.3), 8.30 (1H, d, *J*=2.3), 9.83 (1H, bs); ¹³C-NMR (CDCl₃, δ) 20.1, 38.8, 45.2, 49.4, 124.6, 130.6, 139.0, 149.0, 151.3, 156.1.

2. Biological Assays

Reagent-grade piperonyl butoxide (PB), an inhibitor of oxidative metabolism, is commercially available. NIA16388 (propargyl propyl benzenephosphonate; NIA), an inhibitor of the hydrolytic metabolism of a pyrethroid, tetramethrin,¹⁹⁾ was the same sample used in previous studies.⁷⁻¹⁰⁾

2.1. Insecticidal tests on American cockroaches

The insecticidal test on adult male American cockroaches, *Periplaneta americana* L., was conducted as described previously.⁷⁻¹⁰⁾ In short, a volume (1–10 μl) of a methanol solution of each compound containing some amount of dimethyl sulfoxide (DMSO)

was injected into the abdomen of a cockroach unless otherwise noted. Organic solvents alone in this range did not show any toxic effect. The method of dosing was described previously in detail.⁷⁾ In some experiments, a methanol solution (1 μl) containing PB (50 μg) and NIA (50 μg) was injected 1 hr before injection of the test compounds. The metabolic inhibitors in these amounts did not have any toxic effect. To determine the minimum lethal dose (MLD in mol) for each compound, three insects were used for each dose. They were kept at 22–25°C for 24 hr after the injection. The minimum lethal dose at which two of three insects died was taken as the MLD. Paralyzed insects were counted as dead. The MLD values for the test compounds are listed in Tables 1 and 2.

2.2. Neurophysiological test

The neurophysiological activity of the test compounds was measured in practically the same way as that previously reported.⁷⁻¹⁰⁾ In brief, the excised abdominal central nerve cord of an adult male American cockroach was cut out between the fourth and fifth ganglia. One of two bundles divided from the thoracic side of the nerve cord was tightly taken up with saline (pH 7.3) into a glass tube, in which a chlorinated silver wire was set as the elec-

Table 1. Biological activities of thiamethoxam-type compounds^{a)}

No.	Compound			Insecticidal activity (MLD, nmol) ^{b)}			Neuroblocking activity ^{c)} BC (μM)
	X	Het	R	Alone	+(PB+NIA)	Synergistic effect	
1	O	Thy	H	12	0.59	20	6.0 (5.1–7.1)
2 ^{d)}	O	Thy	Me	1.4	0.35	4	32 (23–38)
3	O	Pyr	H	9.2	0.60	15	20 (19–22)
4	O	Pyr	Me	14	2.3	6	23 (20–30)
5	NMe	Thy	H	180	18	10	82 (71–97)
6	NMe	Thy	Me	11	0.67	16	180 (140–230)
7	NMe	Pyr	H	280	70	4	160 (150–190)
8	NMe	Pyr	Me	98	3.1	32	630 (630–790)
9	S	Thy	H	60	1.9	3	6.5 (5.9–7.8)
10	S	Thy	Me	21	1.3	16	16 (14–18)
11	S	Pyr	H	110	1.4	79	17 (16–17)
12	S	Pyr	Me	14	2.2	6	83 (78–90)
13	CH ₂	Pyr	H	6.1	0.076	80	4.5 (3.6–5.5)
14 ^{e)}				1.1	0.071	15	2.3 (2.0–2.5)

^{a)} Chemical structures are shown in Fig. 1. ^{b)} The value has a deviation of 0.64 to 1.6-fold. ^{c)} Values in parentheses are the deviation range estimated from the concentration-response relationship where each point was determined from more than three runs. ^{d)} Thiamethoxam. ^{e)} Imidacloprid; data from Ref. 8).

Table 2. Biological activities of clothianidin-type compounds^{a)}

Compound			Insecticidal activity (MLD, nmol) ^{b)}			Neuroblocking potency ^{c)} BC (μM)
No.	Het	R	Alone	+(PB+NIA)	Synergistic effect	
15	Thy	H	>400	>400	—	680 (650-720)
16 ^{d)}	Thy	Me	2.0	0.16	13	10 (8.0-10)
17	Pyr	H	>400	>400	—	>650
18	Pyr	Me	5.1	0.51	10	8.5 (7.8-9.6)

^{a)} Chemical structures are shown in Fig. 1. ^{b,c)} See the footnote in Table 1 and the mark > means that the values are larger than those indicated. ^{d)} Clothianidin.

trode. As the reference electrode, another silver wire was set outside the tube. The number of spontaneous discharges that were larger than 15 μV was consecutively counted with a pulse counter (MET-1100, Nihon Kohden, Tokyo) over 30 sec periods. When the frequency decreased and stabilized within a range of 30-400 counts per 30 sec, the saline solution was exchanged for saline containing each test compound dissolved in methanol containing some amount of DMSO. The final concentration of the organic solvents was lower than 1% (v/v), which did not affect the neural activity of compounds. Experiments were conducted at 22-25°C. The concentration for each compound required to block the excitation to a certain level, BC (M), was determined from a dose-response relationship as described previously.⁸⁻¹⁰⁾ The values are listed in Tables 1 and 2.

3. Hydrolysis Measurements

A three ppm SOS solution (NaCl 100 mM, KCl 2.4 mM, CaCl₂ 1.8 mM, and HEPES 5.0 mM, pH=7.6) of each compound was kept at 25°C and an aliquot was injected at 24, 48, 96 or 168 hr into an HPLC system (Jasco UVDECK-100-VI, 254 nm) with an ODS column (Merck, LiChrosorb RP-18) using acetonitrile/water (30:70, v/v) as the mobile phase. The residual amount (%) of the compound is given in Table 3.

RESULTS AND DISCUSSION

The insecticidal activity of compounds 1-12 injected into adult American cockroaches is listed in Table 1 along with that of a hexahydropyrimidine derivative (13) and imidacloprid (14) as references. The effect without a synergist (alone) was dependent on the heteroaromatic ring or the heterocyclic ring conjugated to the nitroimino group. Among the test compounds, thiamethoxam (2) stood out. Its MLD value was as low as 1.4 nmol comparable to that of imidacloprid (14). The insecticidal effects of compounds 1, 3, 4, 6, 10 and 12 were roughly the same as the effect of the prototype (13), while compounds 5, 7-9 and 11 were far less active. Chlorothiazolylmethyl and chloronicotinyl groups appear to be equally effective activators as seen in the pairs 1/3, 5/7 and 10/12, except the pairs 2/4 and 6/8, where the former group evidently excelled. As the heterocycle, an oxadiazine ring was generally more favorable than the thiadiazine and/or triazine rings (1 vs. 5/9; 2 vs. 6/10; 3 vs. 7/11; 4 vs. 8). It has been known

that imidacloprid and its nitromethylene analogues significantly lose the activity by methylation at N3 site of the imidazolidine ring,^{7,20,21)} and the distorted coplanarity of the nitroguanidine (nitromethylene) moiety has been described as defecting the fit with the binding site and hence reducing the activity.^{22,23)} It is of note that for the present compounds except the pair 3/4, a methyl substitution at N3 enhanced the potency. In view of the contrast to other neonicotinoids, a different mode of binding may apply to thiamethoxam and the six-membered heterocyclic analogues tested here.

Pretreatment with synergists (PB+NIA) enhanced the activity of all compounds to various degrees. Recent studies using recombinant cytochrome P450 (CYP450) isoenzymes demonstrated that one of the principal metabolic processes for imidacloprid is reduction at the nitroimine substituent, and that concomitant oxidation at the imidazolidine moiety forming 5-hydroxy and olefin is only a minor path.^{24,25)} This may explain the appreciable synergistic effects of these P450 inhibitors on all the test compounds bearing a nitroimino moiety.

To evaluate the activity at the target site, we measured the effect of the compounds on nerve impulses in a saline solution. Neonicotinoid compounds immediately increase the frequency of impulses in nerve preparations, after which there is a drop to a

Table 3. Residual composition in an SOS solution of 2, 4, 6, 8, 10 and 12 at 25°C at the given hours^{a)}

Compound (%)	Time (hr)				
	0	24	48	96	168
2	100	99	97	98	97
4	100	99	97	96	96
6/16	100/0	88/8	79/12	66/32	58/39
8/18	100/0	86/11	77/20	63/35	46/50
10	100	96	87	79	70
12	100	95	84	80	69

^{a)} See the text for measurement conditions. The data are from one run.

level lower than the control. For a quantitative analysis of the structure-activity relationship including the hydrophobic parameter, the strength of the neuroblocking activity is a more reliable indicator than the nerve excitation effects.^{8,10)} All the compounds tested here blocked nerve impulses at concentrations between 4 and 630 μ M (Table 1). There are a few other remarks in the tests. First, the *N*3-H derivatives had stronger neuroblocking activity than the *N*3-Me derivatives. This tendency is along with imidacloprid and its analogues, conferring a considerable drop in methylation at the corresponding site of the imidazolidine ring.^{7,8)} Second, the triazines were notably less active than the oxadiazines and the thiadiazines. We ascribe the weak neuroblocking activity of the triazine compounds (5–8) to the ionization of the *N*5 nitrogen atom in the saline solution.

The biological tests of clothianidin (16) and the analogous acyclic compounds revealed a distinct difference in activity between *N*3-Me and desmethyl derivatives. Regardless of the attached heteroaromatic ring, the NH compounds (15, 17) were inactive in the insecticidal as well as nerve tests, while the methyl compounds 16 and 18 were highly active in both tests. These results suggest that a lipophilic entity at this site is indispensable for these acyclic neonicotinoids to exhibit activity.

To examine the hydrolytic stability in the outside milieu, we measured the residual amounts of oxadiazines 2 and 4, triazines 6 and 8, and thiadiazines 10 and 12 in a physiological salt solution at 25°C (SOS solution, pH 7.6). Triazines 6 and 8 decayed about 10% to 16 and 18 after 24 hr, while we could not detect any measurable amount of the acyclic compounds from oxadiazines 2 and 4 even after 7 days under these conditions. The decomposition of thiadiazines 10 and 12 was observed, but the ratios of acyclic molecules could not be determined because several decomposed components overlapped in the area of the peak due to the acyclic compound. The sluggish decomposition rates of the oxadiazine compounds compared to the other heterocycles can be ascribed to the larger bond energy for C–O (85–91) than for C–N (69–75) and for C–S (61) bonds in kcal/mol.²⁶⁾

The significantly weak affinity for the [³H]imidacloprid binding sites on insect nAChR from housefly head membrane and absence of pulses in neurons isolated from a lepidopteran species have been the basis for considering thiamethoxam as a prodrug.⁶⁾ The present experiment revealed that although its neuroblocking potency and insecticidal activity with synergists, *i.e.* under the conditions where the effects of metabolic decomposition were limited, were inferior to those for clothianidin, thiamethoxam matched clothianidin in insecticidal activity without synergists. From the results of matching insecticidal activity (alone), there seems no need to consider the prodrug possibility in the present case using American cockroaches.

Triazine compounds 6 and 8 appear different. Their neuroblocking effects were far inferior to those of 16 and 18, but their insecticidal activities were not much weaker. Basic molecules like triazines are ionized in the fluids of insects and reach the synapses only slowly through the lipophilic cuticles and the ion barriers. During retarded movement, the molecule is prone to decompose. This tendency is clearer on estimation of the insecti-

cidal activity 24 hr after injection than estimation of the neuroblocking action at 10 min. We suspect that partial hydrolysis, enzymatically and nonenzymatically, took place and generated nonionizable acyclic molecules that contributed to the insecticidal activity of compounds 6 and 8.

The thiadiazine derivatives showed another biological feature. The insecticidal activities with synergists were very similar for NH (9/11) and N–Me (10/12) compounds as opposed to the other heterocycles. This means that the common framework of $\text{Het-CH}_2\text{NCH}_2\text{-S-CH}_2\text{NR}$ is a target for metabolism and the scission of the labile C–S bonds occurs in several ways, giving various metabolites having different levels of activity and a balance appeared as insecticidal potency without synergist treatment.

We have discussed the biological effect of three quite similar compounds and learned that even a minor structural change, in this case only a single heterorings atom, can bring about a remarkable difference in biological behavior, which suggests that molecular design based on a prodrug concept is not always accord with a prediction.

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Note

Synthesis and Acaricidal Activity of *N*-(1,3,4-Thiadiazol-2-yl)carboxamides

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INTRODUCTION

Some herbicidal *N*-(1,3,4-thiadiazol-2-yl)carboxamides have been reported,^{1,2)} but little is known about their acaricidal activity. In this investigation into the development of a new acaricide, we identified a new class of *N*-(1,3,4-thiadiazol-2-yl)carboxamides with high acaricidal activity.³⁾ This paper describes the synthesis and acaricidal activities of *N*-(1,3,4-thiadiazol-2-yl)carboxamides.

MATERIALS AND METHODS

1. Synthesis of Compounds

Melting points were uncorrected. The structures of compounds were confirmed by ¹H NMR and IR spectra. ¹H NMR spectra were measured on a Varian UNITY 300 spectrometer at 300 MHz using tetramethylsilane as an internal standard. IR spectra were measured on a JASCO IR-810 spectrometer.

N-(1,3,4-Thiadiazol-2-yl)carboxamides were synthesized by the reaction of acid chlorides and 2-amino-1,3,4-thiadiazoles in the presence of a base (Fig. 1). 2-Amino-5-substituted-1,3,4-thiadiazoles were purchased from commercial sources or synthesized according to the reported procedure.^{4,5)} Acid chlorides were purchased from commercial sources or synthesized according to the reported procedure.⁶⁾ 2,2-Dibromo-3,3-dimethylbutanoyl chloride was synthesized by bromination of 3,3-dimethylbutanoyl chloride (Fig. 2).

1.1. 2,2-Dibromo-3,3-dimethylbutanoyl chloride

A mixture of 3,3-dimethylbutanoyl chloride (21.4 g, 159 mmol) and bromine (64.0 g, 400 mmol) was refluxed for 9 hr. Excess bromine was removed, and the residue was distilled

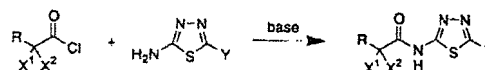


Fig. 1. Synthetic route of *N*-(1,3,4-thiadiazol-2-yl)carboxamides.

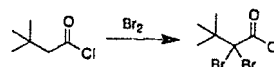


Fig. 2. Synthetic route of 2,2-dibromo-3,3-dimethylbutanoyl chloride.

under reduced pressure to give 18.6 g (40%) of the title compound. bp: 71–72°C (0.6 mmHg); ¹H NMR (CDCl₃) δ (ppm): 1.41 (9H, s); IR (NaCl) cm⁻¹: 2984, 1783, 1464, 1398, 1367, 1041, 1000, 875, 796, 750, 695, 665, 572.

1.2. 2,2-Dibromo-3,3-dimethyl-*N*-(5-pentafluoroethyl-1,3,4-thiadiazol-2-yl)butanamide (23)

A mixture of 2,2-dibromo-3,3-dimethylbutanoyl chloride (8.78 g, 30 mmol), 2-amino-5-pentafluoroethyl-1,3,4-thiadiazole (6.57 g, 30 mmol) and potassium carbonate (4.14 g, 30 mmol) in toluene (100 ml) was refluxed for 6 hr. After cooling to room temperature, the mixture was poured into water (200 ml), acidified with hydrochloric acid and extracted with ethyl acetate. The organic layer was washed with water and saturated aqueous sodium chloride, then dried over sodium sulfate. The solvent was removed under reduced pressure and the resulting solid was washed by hexane to give 23 (9.98 g, 70%). mp: 152–154°C; ¹H NMR (CDCl₃) δ (ppm): 1.33 (9H, s), 11.2 (1H, br).

2. Biological Tests

Test species of mite (*Tetranychus urticae*) and the method used were the same as previously reported.⁷⁾

The activity rating was expressed as indices of 0 to 3, corresponding to 0–29, 30–79, 80–99 and 100% mortality, respectively.

RESULTS AND DISCUSSION

Table 1 shows the effect of substituent (R) of the acid moiety. Only *tert*- and *sec*-alkyl analogs showed a high activity against *T. urticae*. Among these, the *tert*-butyl analog (9) showed activity at 50 ppm. Introduction of a bulkier *tert*-amyl group (10) resulted in reduced activity. The hydrogen (1), *normal*-alkyl (2–4 and 6) and cycloalkyl (11 and 12) analogs showed little or no activity.

Table 2 shows the effect of halogen atoms at 2-position of carbonyl moiety. Halogen atom is essential for acaricidal activity because the nonhalogenated compound (13) did not show any activity at 500 ppm. The dibromo (14) and monochloro (15) analogs were as active as the monobromo analog (9).

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Table 1. Effect of substituent R on activity against *Tetranychus urticae*

No.	R	mp (°C)	Activity rating (<i>Tetranychus urticae</i>)			
			500	200	50	12.5 (ppm)
1	H	150-152	0	- ^{a)}	-	-
2	CH ₃	138-140	0	-	-	-
3	C ₂ H ₅	124-125	0	-	-	-
4	<i>n</i> -C ₃ H ₇	124-126	1	0	-	-
5	<i>i</i> -C ₃ H ₇	131-132	3	1	-	-
6	<i>n</i> -C ₄ H ₉	102-104	0	-	-	-
7	<i>i</i> -C ₄ H ₉	117-119	0	-	-	-
8	<i>s</i> -C ₄ H ₉	108-110	3	3	2	1
9	<i>t</i> -C ₄ H ₉	151-153	3	3	3	1
10	<i>t</i> -C ₅ H ₁₁	155-156	2	2	1	0
11	<i>cyclo</i> -C ₅ H ₉	130-132	1	0	-	-
12	<i>cyclo</i> -C ₆ H ₁₁	148-150	1	0	-	-

^{a)} Not tested.

Table 2. Effect of substituents X¹ and X² on activity against *Tetranychus urticae*

No.	X ¹	X ²	mp (°C)	Activity rating (<i>Tetranychus urticae</i>)			
				500	200	50	12.5 (ppm)
13	H	H	117-119	0	- ^{a)}	-	-
9	Br	H	151-153	3	3	3	1
14	Br	Br	147-149	3	3	3	1
15	Cl	H	162-163	3	3	3	1

^{a)} Not tested.

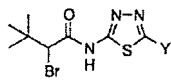
Tables 3 and 4 show the effect of substituent (Y) at 5-position of thiadiazole ring. Unsubstituted (20) and alkyl analogs (21,22) did not show any activity at 500 ppm (Table 3). In the case of monobromo analogs, the trifluoromethyl analog (9) showed the highest activity (Table 3). However, in the case of the dibromo analogs, the pentafluoroethyl (23), heptafluoropropyl (24) and nonafluorobutyl (25) analogs also showed high activity (Table 4). Among these, 2,2-dibromo-3,3-dimethyl-*N*-(5-pentafluoroethyl-1,3,4-thiadiazol-2-yl)butanamide (23) showed the

highest activity.

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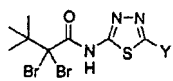
Table 3. Effect of substituent Y on activity against *Tetranychus urticae*



No.	Y	mp (°C)	Activity rating (<i>Tetranychus urticae</i>)				
			500	200	50	12.5	(ppm)
9	CF ₃	151-153	3	3	3	1	
16	CF ₂ CF ₃	201-203	3	2	2	1	
17	(CF ₂) ₂ CF ₃	201-202	2	1	1	1	
18	(CF ₂) ₃ CF ₃	196-197	0	^{a)}	-	-	
19	(CF ₂) ₄ CF ₃	166-167	0	-	-	-	
20	H	212-213	0	-	-	-	
21	CH ₃	217-219	0	-	-	-	
22	C ₂ H ₅	190-192	0	-	-	-	

^{a)} Not tested.

Table 4. Effect of substituent Y on activity against *Tetranychus urticae*



No.	Y	mp (°C)	Activity rating (<i>Tetranychus urticae</i>)					
			500	200	50	12.5	3.13	(ppm)
14	CF ₃	147-149	3	3	3	1	^{a)}	
23	CF ₂ CF ₃	152-154	3	3	3	2	1	
24	(CF ₂) ₂ CF ₃	142-143	3	3	2	0	-	
25	(CF ₂) ₃ CF ₃	89-91	3	3	3	1	-	

^{a)} Not tested.

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Natural products as insecticides: the biology, biochemistry and quantitative structure–activity relationships of spinosyns and spinosoids[†]

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Abstract: The spinosyns, a novel family of insecticidal macrocyclic lactones, are active on a wide variety of insect pests, especially lepidopterans and dipterans. The biological activity of a mixture (spinosad; Tracer[®], Spin-Tor[®], Success[®]) of the two most abundant spinosyns (spinosyns A and D) against pest insects is on a par with that of many pyrethroid insecticides. The spinosyns also exhibit a very favorable environmental and toxicological profile, and possess a mode of action that appears unique, with studies to date suggesting that both nicotinic and *gamma*-aminobutyric acid receptor functions are altered in a novel manner. Compared to pyrethroids such as cypermethrin, spinosyn A is slow to penetrate into insect larvae such as tobacco budworm larvae (*Heliothis virescens*); however, once inside the insect, spinosyn A is not readily metabolized. To date, more than 20 spinosyns and more than 800 spinosoids (semi-synthetic analogs) have been isolated or synthesized, respectively. Artificial neural network-based quantitative structure activity relationship (QSAR) studies for the spinosyns suggested that modification of the 2',3',4'-tri-*O*-methylrhamnosyl moiety could improve activity and several spinosoids incorporating these modifications exhibited markedly improved lepidopteran activity compared to spinosad. Multiple linear regression-based QSAR studies also suggest that whole molecule properties such as CLogP and MOPAC dipole moment can explain much of the biological activity observed for the spinosyns and closely related spinosoids.

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Keywords: insecticide; spinosyn; macrolide; QSAR; artificial neural network; metabolism; tobacco budworm

1 INTRODUCTION

Natural products can be an excellent source of novel chemistries. In the arena of insect control agents, virtually all of the most important insecticidal classes can at least point to a natural product model, if not trace their origins to a natural product. Among the recent insect control agents to reach the marketplace is the natural product, spinosad (Tracer[®], Spin-Tor[®], Success[®]), a naturally occurring mixture of spinosyn A (primary component) and spinosyn D (Fig 1). Spinosyns A and D are members of a new family (Table 1) of fermentation-derived macrolides that are highly active against a variety of insect pests including thrips, fleas and pest species of the Lepidoptera, Diptera and Hymenoptera.^{1–9}

The discovery of the spinosyns was the result of a natural products screening program directed at testing fermentation broth extracts from diverse soil samples for activity against agricultural and pharmaceutical targets. A key component to this program was a miniaturized mosquito larvicide bioassay.¹⁰ In 1985 a

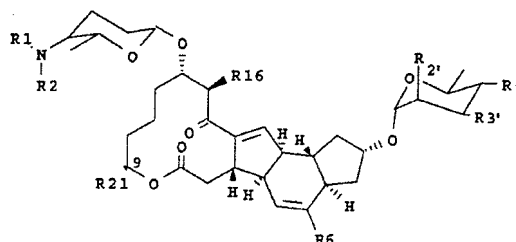


Figure 1. General structure of the spinosyns and spinosoids (see Table 1).

soil sample from the Caribbean was identified as active in the mosquito larvicide bioassay, and the activity was confirmed in laboratory bioassays on southern armyworm larvae.^{4,10} The source of the activity was found to be a new species of actinomycete, *Saccharopolyspora spinosa* Mertz & Yao,¹¹ that produced a family of novel macrocyclic lactones, the spinosyns.^{1,2,4,5,10}

Interest in the spinosyns is due not only to their unique molecular structure, but also to the high levels

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of potency exhibited against agriculturally important pests such as the tobacco budworm, *Heliothis virescens* (F). Against lepidopteran pests such as *H virescens*, the spinosyns exhibit activity on a par with that of members of one of the most insecticidally active classes of molecules, the pyrethroid insecticides (Table 2).²⁻⁵ In a variety of assay systems, the activity of spinosyn A is near to or better than that of pyrethroids such as cypermethrin,⁴ and exceeds the activity of a variety of organophosphorus, carbamate, cyclodiene and other insecticides (Table 2).^{2,4,5,12} Equally important in today's market place is the very favorable environmental and mammalian toxicity profile exhibited by spinosyn A, particularly when compared to

more classical insecticides (Table 2).^{2-7,12} In addition, when compared to pyrethroid insecticides such as cypermethrin, spinosad also exhibits a very favorable profile relative to beneficial insect species.^{6,10}

2 SPINOSYN MODE OF ACTION

Insecticide resistance, and consequently resistance management, has become a primary concern in many insecticide markets due to the ever expanding number of compounds to which many important insect pests are no longer susceptible.¹³ To minimize the potential for cross-resistance, it is highly beneficial for a new insect control agent to possess a novel, or at least an

Table 1. Structures and neonate *Heliothis virescens* larval toxicity for the spinosyns and selected spinosoids

Number	Compound	R1 ^a	R2	R21	R16	R6	R2'	R3'	R4'	LC ₅₀ ^b
Spinosyns										
1	Spinosyn A	Me	Me	Et	Me	H	OMe	OMe	OMe	0.3
2	Spinosyn B	H	— ^c	—	—	—	—	—	—	0.4
3	Spinosyn C	H	H	—	—	—	—	—	—	0.8
4	Spinosyn D	—	—	—	—	Me	—	—	—	0.8
5	Spinosyn E	—	—	Me	—	—	—	—	—	4.6
6	Spinosyn F	—	—	—	H	—	—	—	—	4.5
7	Spinosyn H	—	—	—	—	—	OH	—	—	5.7
8	Spinosyn J	—	—	—	—	—	—	OH	—	>80
9	Spinosyn K	—	—	—	—	—	—	—	OH	3.5
10	Spinosyn L	—	—	—	—	Me	—	OH	—	26
11	Spinosyn M	H	—	—	—	—	—	OH	—	22.6
12	Spinosyn N	H	—	—	—	Me	—	OH	—	40
13	Spinosyn O	—	—	—	—	Me	—	—	OH	1.4
14	Spinosyn P	—	—	—	—	—	—	OH	OH	>64
15	Spinosyn Q	—	—	—	—	Me	OH	—	—	0.5
16	Spinosyn R	H	—	—	—	—	OH	—	—	14.5
17	Spinosyn S	—	—	Me	—	—	OH	—	—	53
18	Spinosyn T	—	—	—	—	—	OH	OH	—	>64
19	Spinosyn U	—	—	—	—	—	OH	—	OH	22
20	Spinosyn V	—	—	—	—	Me	OH	—	OH	17
21	Spinosyn W	—	—	—	—	Me	—	OH	OH	>64
22	Spinosyn Y	—	—	Me	—	—	—	—	OH	20
Spinosoids										
23	N-demethyl D	H	—	—	—	Me	—	—	—	5.6
24	N-demethyl K	H	—	—	—	—	—	—	OH	9.9
25	N,N-didemethyl K	H	H	—	—	—	—	—	OH	7.4
26	N-demethyl P	H	—	—	—	—	—	OH	OH	>64
27	2'-H A	—	—	—	—	—	H	—	—	0.23
28	2'-H D	—	—	—	—	Me	H	—	—	0.23
29	2'-O-ethyl A	—	—	—	—	—	OEt	—	—	0.30
30	3'-H A	—	—	—	—	—	—	H	—	0.36
31	3'-O-ethyl A	—	—	—	—	—	—	OEt	—	0.03
32	3'-O-n-propyl A	—	—	—	—	—	—	OnPr	—	0.05
33	3'-O-n-butyl A	—	—	—	—	—	—	OnBu	—	0.38
34	3'-O-allyl A	—	—	—	—	—	—	OAllyl	—	0.06
35	3'-O-CH ₂ CF ₃ A	—	—	—	—	—	—	OCH ₂ CF ₃	—	0.43
36	4'-H A	—	—	—	—	—	—	—	H	0.41
37	4'-O-ethyl A	—	—	—	—	—	—	—	OEt	0.24
38	2',3',4'-tri-O-ethyl A	—	—	—	—	—	OEt	OEt	OEt	0.02
	Cypermethrin									0.18

^a Position on the spinosyn structure (see Fig 1).
^b *Heliothis virescens* neonate larval bioassay; LC₅₀ in mg litre⁻¹.
^c dash = no change from substitution pattern for spinosyn A.

1-2 Me-H ↑
1-4 H-Me ↑
1-5 M-H ↑
1-6 Me-H ↑
1-7 Me-H ↑
1-8 Me-H ↑
1-9 Me-H ↑

under-exploited, mode of action. In addition to a unique chemical structure, available data suggest that the spinosyns also possess a novel mode of action. The spinosyns cause hyperexcitation, and ultimately disruption, of the insect central nervous system.¹⁴⁻¹⁶ Electrophysiological evidence indicates that the spinosyns can alter nicotinic currents in neuronal cell bodies from the central nervous system of the American cockroach (*Periplaneta americana* (L)).¹⁴ This nicotinic effect is correlated with toxicity to neonate *H virescens* larvae.¹⁴ In addition to altering nicotinic function, electrophysiological evidence also indicates that spinosyns can disrupt the function of gamma-aminobutyric acid (GABA) receptors of small neurons from the central nervous system of *P americana*. Insecticidal spinosyns and spinosoids disrupt GABA receptor function in these neurons, while spinosyns lacking insecticidal activity do not. The initiation of a relatively small-amplitude chloride conductance by the insecticidal spinosyns coincides with this loss of GABA receptor responses, and may underlie the loss of receptor function (Gerald Watson, Dow AgroSciences, pers comm). However, spinosyns and spinosoids have not been found to affect the binding of either nicotinic or GABA receptor radioligands¹⁴ (Nailah Orr, Dow AgroSciences, pers comm), suggesting that the spinosyns do not interact directly with known nicotinic or GABA receptor binding sites. These results suggest that the spinosyns can affect nicotinic/GABA receptors through an undetermined mechanism that may underlie the insecticidal properties of the spinosyns and their semi-synthetic analogs (spinosoids).

3 STRUCTURE-ACTIVITY RELATIONSHIPS (SAR)
3.1 Spinosyn SAR

In the light of the excellent insecticidal properties of spinosyn A and spinosad, as well as their novel mode of action, a program was undertaken, initially by Lilly Research Laboratories (LRL), and then by Dow AgroSciences, to explore spinosyn chemistry further so as to both expand the spectrum and increase insecticidal potency. This program consisted of preparing semi-synthetic derivatives/analogues of the spinosyns, termed spinosoids,^{9,12,17} as well as the continued isolation and identification of new spinosyns (naturally occurring analogues of spinosyn A).^{1,3,5,8} More than 20 spinosyns have been isolated (Table 1).^{3,5,8} Spinosyn A is highly active against neonate larvae of the tobacco budworm, *H virescens*, (Tables 1 and 2) as are spinosyns B, C, D and Q (Table 1).^{2,3,5,8} As demonstrated by spinosyns E, F, S and Y, a reduction in the size of the alkyl group at C16 or C21 (Fig 1; Table 1) reduces *H virescens* activity. With the exception of spinosyn Q, loss of a methyl group from the rhamnose moiety at the 2'-position (spinosyn H and its analogs, Q, R, S), 3'-position (spinosyn J and its analogs L, M, N) or 4'-position (spinosyns K and its analogs O, Y) all result in a decrease in activity towards *H virescens* larvae when compared to spinosyn A. The effect of losing this methyl group is greatest at the 3'-position, where activity decreases, in some cases, by more than two orders of magnitude (Table 1). The di-demethyl rhamnosyl spinosyns (eg spinosyns P, U, V and W) are only weakly active at best, with those lacking a 3'-methyl group being nearly inactive (Table 1).^{2,5} As noted previously^{2,3,5,8,9} these data clearly demonstrate

Table 2. Toxicity and penetration profiles (technical material) of spinosyn A compared to selected insecticides

Assay/test	Spinosyn A	Cypermethrin	Permethrin ^a	Parathion-methyl	Methomyl
<i>Heliothis virescens</i> (LD ₅₀ or LC ₅₀)					
3rd instar, topical (µg g ⁻¹)	1.28-2.25	0.241-1.61	1.33-2.79	11.6-65.0	4.33, 26.7
2nd instar, contact (mg litre ⁻¹)	16.41	3.21	—	—	—
Neonate drench (mg litre ⁻¹)	0.26	0.18	—	—	—
2nd instar, leaf dip (mg litre ⁻¹)	1.02	4.06	—	—	—
2nd instar, leaf spray (mg litre ⁻¹)	1.93	4.20	—	—	—
5th instar, injection (µg g ⁻¹)	0.75	0.84	—	—	—
5th instar, topical (µg g ⁻¹)	4.08	0.83	—	—	—
<i>Heliothis virescens</i> (3rd instar)					
% Penetration (h)	2.3 (3)	35-36 (3)	9-18 (2-3)	28.9 (4)	—
<i>Trichoplusia ni</i> (5th instar)					
% Penetration (h)	2.1-7.5 (3-4)	30.8 (3)	33.2 (4)	—	—
Non-target tests (LD ₅₀ or LC ₅₀)					
Rat oral (mg kg ⁻¹)	3783-5000	247	>4000	9	17
Rat dermal (mg kg ⁻¹)	>2000	>2000	—	—	—
Rabbit dermal (mg kg ⁻¹)	—	>2000	>4000	63	1000
Rabbit skin irritation	Non irritant	Moderate irritant	—	—	—
Mallard duck, acute oral (mg kg ⁻¹)	>2000	>10000	—	—	—
Quail, acute oral (mg kg ⁻¹)	>2000	—	—	—	—
Rainbow trout, acute 96h (mg litre ⁻¹)	30	0.025	—	—	—
Carp, acute 96h (mg litre ⁻¹)	96	0.0016	—	—	—
<i>Daphnia magna</i> , 48h (mg litre ⁻¹)	96	0.0013	—	—	—

Data adapted, in part, from Sparks *et al*, References 2, 4, 5, 28.
^a 40:60 *cis:trans* mixture.

that relatively small changes in the spinosyn structure are often associated with large changes in biological activity. Although spinosyn A is slightly more active than spinosyn D, in general spinosyns possessing a methyl group at C6 (spinosyn D-related analogs) tend to be more active and less affected by changes in the rest of the molecule⁵ than the corresponding analogs possessing only a hydrogen at C6 (Table 1).

3.2 Application of artificial neural networks to spinosyn SAR

Starting early in the spinosyn discovery program, and running alongside the continuing search for new, naturally occurring spinosyns, several hundred semi-synthetic derivatives of the spinosyns (spinosoids) were prepared. All of the early spinosoids were typified by a reduced or even total lack of *H. virescens* activity compared to spinosyn A. The one exception was the de-methoxy (2'-H) analogs of spinosyns H and Q (see Table 1), prepared sometime into the program, which were as active as, or slightly more so, than spinosyn A¹⁸ (Table 1). These spinosoids were the first indication that semi-synthetic analogs could be at least as active as the best of the spinosyns (ie spinosyn A). As an adjunct to the synthetic efforts in the early discovery program, computer modeling studies of the spinosyns/spinosoids was conducted as a means of identifying potential synthetic directions. However, the large molecular size of the spinosyns (molecular mass = 732), their complex chemical structure (Fig 1) and limited computing power all contributed to difficulties in understanding, at that time, the parameters that governed the biological activity. Shortly afterwards, an alternative approach to spinosyn quantitative structure activity relationships (QSAR) was taken in the form of artificial neural networks (ANN). ANN are usually software-based systems that are modeled on concepts derived from the mammalian nervous system.^{19,20} In contrast to multiple linear regression (MLR) and other conventional QSAR methods, ANNs are fundamentally learning machines that model a particular problem by attempting to learn from the data, so deriving rules or relationships. As such, ANNs are good with data that is incomplete, noisy or non-linear, and studies suggested that ANNs could provide a viable alternative to classical QSAR methodologies.¹⁹⁻²² ANN-based QSAR studies of the spinosyns/spinosoids¹⁷ suggested several possible approaches to improve activity, one of which involved extending the alkyl chain of the 2', 3' and 4'-O-methyl groups. Subsequent synthesis and testing of the 2'-3'-4'-tri-O-ethyl analog of spinosyn A clearly demonstrated a marked improvement in activity (3.8 to 17-fold, see also Table 1) against *H. virescens* larvae. Further ANN analysis and synthesis pinpointed the 3'-position as the most critical for modification in the rhamnose moiety¹⁷ (see also Table 1).

3.3 Spinosyn and spinosoid spectrum

The ANN-derived directions for the spinosoids led to

further spinosoid synthesis. The spinosyns exhibit activity against a variety of insect species, especially lepidopterans and dipterans.^{2,6} As noted in the ANN analysis, modifications to the 3'-position of the rhamnose moiety have the greatest effect on activity towards *H. virescens* larvae (Table 1). Extending the alkyl chain from methyl to ethyl at the 2' or 4' positions (Table 1, compounds 29 and 37) produces no improvement in activity compared with spinosyn A. In contrast, the 3'-O-ethyl analog of spinosyn A (Table 1, compound 31) is about ten-fold more active than spinosyn A (Table 1, compound 1). Further extension of the 3'-O-alkyl chain to propyl, allyl or butyl results in no improvement in activity compared with the 3'-O-ethyl derivative (Table 1), and with the chain length reaching four carbons (butyl), there is a distinct decline in activity. Likewise, the presence of fluorine atoms on the 3'-O-alkyl group (ie 3'-O-CH₂CF₃) reduces activity (Table 1). Thus, for the 3'-position of the rhamnose moiety, there appears to be an alkyl chain length optimum at around two to three carbons.

The spinosyns also possess activity against two-spotted spider mites, *Tetranychus urticae* Koch, but the level of activity is marginal compared to commercial standards.⁵ Among the spinosyns tested against *T. urticae*, spinosyns K and O (both 4'-OH) are the most acutely active.^{5,9} Compared to spinosyn K, several 3'-O-alkyl analogs exhibit equivalent acute activity against *T. urticae*, while some modest improvements in acute activity were noted for those analogs possessing a reduced 5,6 double bond.⁹ In addition, residual activity is a critical feature for a good acaricide, and the spinosyns/spinosoids lack this in comparison to current standards.⁹ Likewise, the spinosyns also exhibit only moderate acute activity towards aphids such as the cotton aphid, *Aphis gossypii* Glover.^{5,8,9} The aphid activity is improved, to some degree, with the 3'-O-alkyl analogs,⁹ but the improvements in activity observed are not sufficient for commercial application. Thus, the 3'-O-alkyl analogs identified by the ANN QSAR are able, to varying degrees, to improve the activity of the spinosoids towards several species.^{9,17}

Although the ANN QSAR studies were able to provide very useful synthetic directions, due to the nature of ANNs it is difficult to understand the physicochemical basis for the suggested modifications/improvements. As such, a classical Hansch type multiple linear regression (MLR) analysis^{23,24} was again undertaken as a means of providing insights into the molecular properties that appear to explain spinosyn SAR towards neonate *H. virescens* larvae. A summary of the results from initial²⁵ and subsequent studies follows.

4 MATERIALS AND METHODS

4.1 Penetration and metabolism

A key parameter in the activity of an insecticide is its concentration at the target site as a function of time.

By injection, spinosyn A is as active as cypermethrin (Table 2).⁴ However, when applied topically, spinosyn A is less active than cypermethrin (Table 2). These data suggest that spinosyn A may be penetrating into the larvae at a rate slower than that of smaller, more lipophilic insecticides such as cypermethrin. Thus a series of studies were undertaken to investigate the relative rates of penetration and metabolism of spinosyn A.

Technical (95–99%) and radiolabeled (2'-O-methyl C¹⁴, 50mCi mmol⁻¹) samples of spinosyn A and technical (95–99%) samples of spinosyns B and J were from Dow AgroSciences. Third and last (day 2) stadium *H virescens* and last stadium day 3, cabbage looper (*Trichoplusia ni* Huebner) larvae were reared on an artificial diet at 27 (+2) °C, 50% RH, with a 14:10 h L:D photoperiod. Larvae (four to nine per time point, each in a 20-ml vial) were topically treated with radiolabeled material in acetone (1 µl per larva). At selected intervals following treatment, the larvae from each vial were rinsed with acetone (2 × 2 ml), the solvent evaporated and the remaining radioactivity quantified by liquid scintillation counting (LSC). The *H virescens* larvae were homogenized in 500 µl (penetration) or 1000 µl (metabolism) methanol, while *T ni* larvae had hemolymph removed.²⁶ For the penetration studies, 100 µl of the methanolic homogenate (*H virescens*) or an aliquot of the hemolymph (*T ni*) was quantified by LSC. For metabolism studies the centrifuged (800g, 10 min) methanolic homogenate (reduced to 100 µl under a stream of nitrogen) was analyzed by HPLC (Waters and Associates) using a Sepelco 15 cm, C18 column eluted with methanol + water at 1 ml min⁻¹. Parent and metabolite quantification was by UV and radiochemical detection. Radioactivity remaining in the holding vial was also quantified by LSC. Topical bioassays were conducted as described previously.⁴ Injection assays (1 µl in acetone via a 10-µl Hamilton syringe) were conducted using day 2, last stadium *H virescens* larvae.

4.2 Spinosyn/spinosoid MLR-based QSAR

Heliothis virescens LC₅₀ data for the spinosyns and spinosoids used (Table 3) in the analysis was taken, in part, from previous studies.^{3,5,9} Multiple regression analysis of neonate *H virescens* larval toxicity for a set of spinosyns (1–17, 22) or spinosyns/spinosoids (1–17, 22–38; Table 3) was carried out on a personal computer system using Molecular Analysis Pro 2.0 (WindowChem Software). The X-ray crystal structure for spinosyn A was the starting point for generating the three-dimensional structure for all of the other spinosyns and their respective whole-molecule properties [eg using MOPAC, (a general-purpose semi-empirical molecular orbital package for the study of molecular structures) dipole, HOMO (highest occupied molecular orbital)/LUMO (lowest unoccupied molecular orbital)]. The whole-molecule properties were calculated using either TSAR 2.31 (Oxford Molecular Ltd) on a Silicon Graphics System or

Molecular Analysis Pro 2.0 on a personal computer system after minimization via SYBYL 6.3 (Tripos Inc, St Louis, MO) or Molecular Modeling Pro 1.2 (WindowChem Software), respectively.

5 RESULTS AND DISCUSSION

5.1 Penetration and metabolism

The present *in vivo* studies show that spinosyn A penetrates only slowly into *H virescens* larvae. Compared to available data for other insect-control agents, spinosyn A is one of the slowest in its speed of penetration during the first few hours following application (Table 2).²⁷ Likewise, the penetration of spinosyn A into last stadium *T ni* larvae is also far slower than that of cypermethrin or permethrin (Table 2). Coupled with its slow rate of penetration, spinosyn A is poorly metabolized in *H virescens* larvae. HPLC chromatograms detected no trace of any metabolite, including spinosyns J and B, two of the most likely metabolites.²⁷ An examination of the total internal radioactivity and internal parent (spinosyn A) showed no significant differences (Fig 2). Other studies involving injection of radiolabeled spinosyn A into tobacco budworm larvae or incubation of spinosyn A *in vitro* with midgut microsomes also revealed no significant metabolism²⁷ (Joel Sheets, Dow AgroSciences, pers comm). These observations are further supported by synergist studies. Piperonyl butoxide (an inhibitor of monooxygenases) can synergize permethrin, but not spinosyn A in house flies.²⁷ Likewise, no synergism of spinosyn A was noted in *H virescens* larvae,²⁷ nor is there any evidence suggesting that bioactivation of the spinosyns is required for activity.²⁷ Thus, available data suggest that, although spinosyn A is slow (relative to cypermethrin) to penetrate into *H virescens* larvae, once inside it is not readily metabolized. This apparent lack of metabolism may, in part, compensate for the slow rate of penetration and, thereby, contribute to the relatively high level of activity observed for spinosyn A. Additionally, the apparent lack of metabolism simplifies, to a degree, the interactions of spinosyn A and the spinosyns with the insect. Available data suggest that there is a good correlation between neonate *H virescens* toxicity and the ability of the spinosyns/spinosoids to alter the function of at least one of the systems perturbed by the spinosyns.^{9,14} As such, spinosyn toxicity to neonate *H virescens* may, to a large degree, reflect action at the target site and thus improve the chances for relatively simple whole-molecule properties to explain the biological activity.

5.2 Spinosyn/spinosoid MLR-based QSAR

A variety of whole-molecule properties, including molar refractivity (MR), molecular volume, molecular length, width and depth, CLogP (calculated log P), CLogP,² total dipole, MOPAC dipole (whole molecule dipole moment), HOMO, LUMO, molecular weight, surface area, hydrogen bond donor/acceptor and ellipse volume parameter (Table 3), were exam-

Table 3. Whole molecule properties of the spinosyns

	<i>Hv</i> <i>LC50</i>	<i>Log10</i> <i>LC50</i>	<i>MOPAC</i> <i>dipole</i>	<i>Total</i> <i>dipole</i>	<i>HOMO</i>	<i>LUMO</i>	<i>MW</i>	<i>CLogP</i>	<i>CLogP</i> ²	<i>MR</i>	<i>Mol</i> <i>vol</i>	<i>Ellipsoid</i> <i>vol</i>	<i>Surface</i> <i>area</i>	<i>Molecular</i> <i>width</i>	<i>Molecular</i> <i>length</i>	<i>Molecular</i> <i>depth</i>	<i>H Bond</i> <i>acceptor</i>	<i>H bond</i> <i>donor</i>	<i>4°NR</i>
1 Spinosyn A	0.31	-0.509	1.143	1.218	-9.213	-0.137	732	3.733	13.93	197.7	584.5	4244.8	55.353	13.795	22.229	9.382	1.798	0.000	2
2 Spinosyn B	0.36	-0.444	1.382	1.212	-9.549	-0.146	718	3.371	11.37	192.4	570.7	3840.9	53.966	13.795	22.136	8.888	2.000	0.071	1
3 Spinosyn C	0.82	-0.086	0.750	0.555	-9.686	-0.139	704	2.963	8.78	187.6	557.5	3376.1	52.562	13.705	22.136	8.888	2.212	0.161	0
4 Spinosyn D	0.93	-0.032	1.163	1.115	-9.209	-0.121	746	3.886	15.10	202.0	597.5	4112.4	56.333	13.826	22.184	9.377	1.772	0.000	2
5 Spinosyn E	4.60	0.660	1.112	1.184	-9.214	-0.137	718	3.264	10.65	193.1	570.9	4059.0	54.026	13.795	22.229	9.382	1.798	0.000	2
6 Spinosyn F	4.50	0.650	0.885	0.935	-9.210	-0.142	718	3.170	10.05	193.1	571.9	4079.1	54.068	13.795	22.229	9.382	1.798	0.000	2
7 Spinosyn H	3.20	0.505	1.947	2.184	-9.219	-0.155	718	3.454	11.93	192.9	570.5	4015.1	53.903	13.795	22.229	9.382	2.010	0.127	2
8 Spinosyn J	64.00	1.806	2.620	2.078	-9.208	-0.131	718	3.454	11.93	192.9	571.8	4158.0	54.017	13.795	21.698	9.382	2.010	0.126	2
9 Spinosyn K	1.01	0.004	1.024	1.858	-9.217	-0.149	718	3.454	11.93	192.9	571.3	4353.5	53.948	13.795	22.229	9.377	1.983	0.126	2
10 Spinosyn L	26.00	1.140	2.756	2.109	-9.205	-0.116	732	3.608	13.02	197.2	584.9	4333.0	54.974	13.826	21.672	9.377	1.983	0.126	2
11 Spinosyn M	22.60	1.354	2.950	2.201	-9.544	-0.139	704	3.093	9.57	187.6	558.4	3878.0	52.625	13.795	21.605	8.888	2.211	0.198	1
12 Spinosyn N	12.70	1.104	3.096	2.273	-9.386	-0.124	718	3.246	10.54	191.9	571.4	4032.7	53.593	13.826	21.595	8.888	2.185	0.198	1
13 Spinosyn O	1.40	0.146	1.027	1.824	-9.214	-0.134	732	3.608	13.02	197.2	584.1	4226.2	54.919	13.826	22.184	9.377	1.963	0.126	2
14 Spinosyn P	64.00	1.806	2.121	2.514	-9.214	-0.148	704	3.176	10.09	188.2	558.6	4266.6	52.607	13.795	21.123	9.382	2.221	0.253	2
15 Spinosyn Q	0.39	-0.409	1.931	2.035	-9.216	-0.139	732	3.608	13.02	197.2	583.3	3902.4	54.937	13.826	22.184	9.377	1.963	0.127	2
16 Spinosyn R	14.50	1.161	2.038	1.862	-9.396	-0.176	704	3.093	9.57	187.6	557.2	3620.9	52.511	13.795	22.136	8.888	2.211	0.198	1
17 Spinosyn S	64.00	1.806	1.941	2.196	-9.221	-0.155	704	2.986	8.91	188.4	557.1	3797.8	52.571	13.795	22.229	9.382	2.010	0.127	2
22 Spinosyn Y	20.00	1.301	0.991	1.811	-9.218	-0.149	704	2.986	8.91	188.4	557.6	3782.6	52.616	13.795	22.229	9.382	2.010	0.126	2
23 <i>N</i> -demethyl D	5.60	0.748	1.701		-9.386	-0.143	732	3.525	12.42										1
24 <i>N</i> -demethyl K	9.90	0.996	2.363		-9.393	-0.171	706	3.093	9.57										1
25 <i>N,N</i> -didemethyl K	7.40	0.869	1.297		-9.706	-0.162	692	2.685	7.21										0
26 <i>N</i> -demethyl P	64.00	1.806	2.638		-9.392	-0.168	692	2.815	7.92										1
27 2'-H A	0.23	-0.638	1.933		-9.209	-0.117	704	4.069	16.56										2
28 2'-H D	0.23	-0.638	1.949		-9.206	-0.102	716	4.222	17.83										2
29 2'-OEt A	0.30	-0.523	1.131		-9.211	-0.132	744	4.075	16.61										2
30 3'-H A	0.36	-0.444	1.652		-9.207	-0.112	704	3.879	15.05										2
31 3'-OEt A	0.03	-1.523	1.001		-9.210	-0.129	744	4.418	19.52										2
32 3'-Opropyl A	0.05	-1.301	0.829		-9.209	-0.107	756	4.620	21.34										2
33 3'-Obutyl A	0.38	-0.420	0.819		-9.209	-0.109	768	5.150	26.52										2
34 3'-Oallyl A	0.06	-1.222	1.102		-9.214	-0.124	754	4.130	17.06										2
35 3'-OCH ₂ CF ₃ A	0.43	-0.367	3.228		-9.236	-0.168	798	4.850	23.52										2
36 4'-H A	4.10	0.613	2.087		-9.392	-0.149	704	3.879	15.05										2
37 4'-OEt A	0.24	-0.620	1.238		-9.215	-0.121	744	4.090	16.73										2
38 2',3',4'-tri-OEt A	0.02	-1.699	1.018		-9.211	-0.128	768	4.760	22.66										2

Spinosyn and spinoside as insecticides

TC Sparks, GD Crouse, G Durst

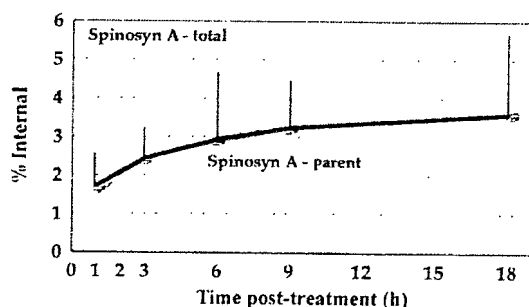


Figure 2. Metabolism (total and parent) of topically applied spinosyn A (1 µg per larva) in last stadium, day 2 *Heliethis virescens* larvae. Data points are means (bar=standard deviation).

ined for their ability to explain the activity of the spinosyns on *H virescens* larvae. The biological response to the modeled spinosyns was best described by eqn (1). The three whole-molecule parameters included were CLogP, HOMO and MOPAC dipole (dipole moment for the spinosyn).

$$\log LC_{50} = -2.18 \text{ CLogP} + 2.89 \text{ HOMO} + 0.61 \text{ MOPAC dipole} + 33.74 \quad (1)$$

($r^2=0.824$, $s=0.372$, $F<0.0001$, $q^2=0.724$, $n=18$).

Equation (1) provides a reasonable ($r^2=0.824$), cross-validated ($q^2=0.742$) explanation of the biological activity of the spinosyns against neonate *H virescens* larvae. None of the three parameters in eqn (1) is highly correlated with another, and individually no one of the three parameters provided a good explanation for the observed biological activity.²⁵ Likewise, no combination of any two of these parameters [see eqns (2)–(4)] is sufficient to provide a good explanation of the observed *H virescens* activity. However, eqn (3) provided the highest r^2 value (0.593) of the three eqns (2)–(4), indicating that the combination of CLogP and MOPAC dipole moment is able to explain more of the data than any other combination of two parameters.

$$\log LC_{50} = -2.23 \text{ CLogP} + 2.73 \text{ HOMO} + 33.46 \quad (2)$$

($r^2 = 0.488$, $s = 0.612$, $F = 0.0065$, $n = 18$)

$$\log LC_{50} = -1.46 \text{ CLogP} + 0.58 \text{ MOPAC dipole} + 4.55 \quad (3)$$

($r^2 = 0.593$, $s = 0.545$, $F = 0.0012$, $n = 18$)

$$\log LC_{50} = 1.07 \text{ HOMO} + 0.63 \text{ MOPAC dipole} + 9.57 \quad (4)$$

($r^2 = 0.382$, $s = 0.673$, $F = 0.027$, $n = 18$)

Interestingly, if the data set is restricted to only analogs possessing the 4"-N,N-dimethyl moiety (ie removal of

spinosyns B, C, N, M, R), then the value of r^2 greatly improves for the two-parameter equation of CLogP and MOPAC dipole, and produces a significant cross-validation index [eqn (5)].

$$\log LC_{50} = -2.11 \text{ CLogP} + 0.70 \text{ MOPAC dipole} + 6.78 \quad (5)$$

($r^2 = 0.785$, $s = 0.422$, $F = 0.00046$, $q^2 = 0.646$, $n = 13$)

The results obtained with eqn (5) suggests that HOMO in eqn (1) may be closely associated with the substitution pattern of the 4"-forosamine nitrogen. This appears to be the case, since replacing HOMO with a simple indicator variable for the number of methyl groups attached to the forosamine nitrogen (NR), produces a significant, cross-validated equation, eqn (6), and there is a highly significant relationship between HOMO and NR (eqn (7)).

$$\log LC_{50} = -2.19 \text{ CLogP} + 0.62 \text{ MOPAC dipole} + 0.71 \text{ NR} + 5.73 \quad (6)$$

($r^2 = 0.809$, $s = 0.387$, $F < 0.0001$, $q^2 = 0.683$, $n = 18$)

$$\text{HOMO} = 0.24 \text{ NR} - 9.70 \quad (7)$$

($r^2 = 0.934$, $s = 0.040$, $F < 0.0001$, $q^2 = 0.911$, $n = 18$)

In the light of the relationship between HOMO and substitution on the forosamine nitrogen, a great deal of *H virescens* activity for the spinosyns modeled can be explained by CLogP and MOPAC dipole moment. In eqns (1), (3), (5) and (6), CLogP is always associated with a negative number, suggesting that enhanced biological activity is generally associated with greater lipophilicity.²⁵ In contrast, the more active spinosyns tend to have smaller MOPAC dipole moments. A plot of MOPAC dipole versus CLogP generates three broad groups for the spinosyns (Fig 3). The first group is composed of spinosyn J and its analogs (L, M, N), which have the largest dipoles and tend to be the least active (Fig 3). Spinosyn H, its analogs (Q, R, S) and spinosyn P comprise the second group, which is characterized by somewhat smaller dipole moments and (with the exception of spinosyn P) better overall activity towards *H virescens* larvae compared to the spinosyn J group. The third group is made up of spinosyn A, its analogues (B, C, D, E and F), along with spinosyn K and its analogues (O, Y). Members of this third group all have comparatively smaller dipole moments (Fig 3) and, in general, possess moderate to good activity against *H virescens* larvae (Table 1).

The present studies suggest, as a general trend for this data set, that dipole moment is largely a function of the rhamnose ring substitution pattern, which is

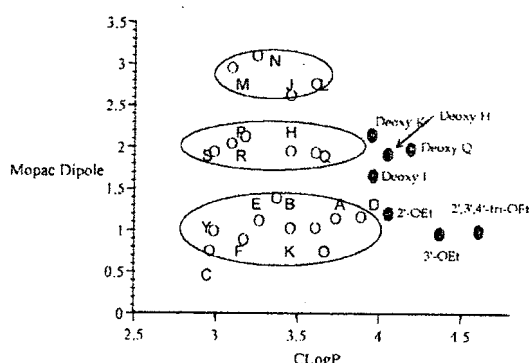


Figure 3. Plot of CLogP versus Mopac dipole moment for selected spinosyns and spinosoids. The values for the spinosyns cluster into three groups (see text for details) while the values for the spinosoids fall into regions that are expected to be more biologically active than their corresponding spinosyn counterparts (see text for details).

secondarily influenced by the forosamine nitrogen substitution pattern. The present studies also suggest that the combination of CLogP and MOPAC dipole can provide insights into spinosyn activity. For example, the available data suggest that *H virescens* activity of the spinosyns might be improved by increasing the relative CLogP while minimizing the MOPAC dipole moment. If the CLogP and MOPAC dipole moments of spinosoids such as the 2',3',4'-triethoxy and 3'-ethoxy analogs of spinosyn A (Tables 1 and 3) are calculated and plotted, they clearly fall in the region expected to be more active than spinosyn A (Fig 3). Likewise, the enhanced activity of the 2'-H, 3'-H and 4'-H analogs (deoxy analogs) of spinosyns H, Q, J and K, compared to their parents, is also explained by the analogs' corresponding shifts in MOPAC dipole and CLogP (Fig 3). Obviously, there will be exceptions to such simple relationships, and it is reasonable to expect that there are optima for each of the above parameters beyond which activity declines. In the case of the 3'-position of the rhamnose, extension of the alkyl group from 3'-ethoxy to 3'-propoxy, 3'-allyloxy or especially 3'-butoxy results in a decrease in activity (Table 1). This apparent optimum in 3'-alkyl chain length is reflected by a reduction in the regression coefficient (r^2) and the cross-validation index (q^2) for an MLR equation using the same parameters as equation (1) and encompassing a larger dataset ($n = 34$) that includes the 3'-alkoxy spinosoids mentioned above [equation (8)].

$$\begin{aligned} \log \text{LC}_{50} = & -1.33 \text{ CLogP} + 2.00 \text{ HOMO} \\ & + 0.59 \text{ MOPAC dipole} + 22.77 \end{aligned} \quad (8)$$

$$(r^2 = 0.758, s = 0.535, F = < 0.0001, q^2 = 0.671, n = 34)$$

One aspect that may not be adequately captured by the

above equation is the observed optimum of two to three carbons for alkyl length at the 3'-position of the rhamnose moiety (Table 2). Since the CLogP parameter appears to be primarily associated with the rhamnose moiety, it is likely that addition of a squared term for the CLogP parameter (CLogP²) to eqn (8) will produce an equation that provides a better fit for the data and better reflects the presence of a non-linear relationship^{23,24} at the 3'-position. As shown by eqn (9), this is the case. The addition of the squared term of ClogP improved (r^2 and q^2 increased from 0.758 to 0.816, and from 0.671 to 0.706, respectively) the ability of the resulting eqn (9) to explain the activity of the spinosyns/spinosoids towards neonate *H virescens* larvae.

$$\begin{aligned} \log \text{LC}_{50} = & -6.62 \text{ CLogP} + 0.67 \text{ CLogP}^2 \\ & + 3.09 \text{ HOMO} + 0.59 \text{ MOPAC dipole} + 43.05 \end{aligned} \quad (9)$$

$$(r^2 = 0.816, s = 0.475, F = < 0.0001, q^2 = 0.706, n = 34)$$

The present investigation of the QSAR for sets of spinosyns and spinosoids suggests that certain whole-molecule properties appear to be well correlated with the biological activity of these novel molecules towards larvae of *H virescens*. As such, the mathematical relationships of these properties have been useful in attempting to understand the physicochemical parameters that are associated with the biological activity of spinosyns and spinosoids. In addition, these relationships may also be used, within limits, to predict the activity of other molecules within the series.

6 SUMMARY

Available data^{12,27} (Table 2, Fig 2) suggest that, compared to a variety of insecticides including pyrethroids such as cypermethrin, spinosyn A is slow to penetrate into larvae of *H virescens*. However, once inside the insect, spinosyn A does not seem to be readily metabolized. Thus, the apparent lack of spinosyn A metabolism by *H virescens* larvae may contribute to the high level of activity noted for the spinosyns and, in part, compensate for the slow rate of penetration.

Several hundred spinosoids have been synthesized and ANN-based QSAR studies have, in part, been used to guide some of the syntheses.¹⁷ One ANN-based modification proposed to improve activity involved the 2',3',4'-tri-*O*-methylrhamnosyl moiety (ie extension of the alkyl chain from methyl to ethyl) and several spinosoids incorporating these modifications exhibit markedly improved lepidopteran activity compared to spinosad. A number of MLR-based QSAR studies also suggest that whole-molecule properties such as CLogP and MOPAC dipole

moment can explain much of the neonate *H virescens* biological activity observed for the spinosyns and closely related spinosoids. In general, among the spinosyns/spinosoids examined, those possessing a lower MOPAC dipole and a higher CLogP tend to be the more active. Although the utility of these parameters is likely to change with the bioassay system used, the above relationships may be useful as a reference point in attempting to understand the biological activity of spinosyns and spinosoids.

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Original Article

Synthesis and Structure-Activity Relationships of Dinotefuran Derivatives: Modification in the Tetrahydro-3-furylmethyl Part

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The ($\pm$)-tetrahydro-3-furylmethyl moiety, which is a characteristic part of the novel neonicotinoid dinotefuran, was found by research in which acetylcholine was selected as the lead compound. SAR (structure-activity relationships) for the tetrahydrofuran part indicated that the non-substituted moiety showed the highest level of activity, 4- and 5-substituted moieties showed intermediate levels, and 2- and 3-substituted moieties lost the activity. Conformational analysis of these compounds indicated that the substituents changed little the hypothetical active conformation of dinotefuran. Computational analysis proved that dinotefuran, a methoxypropyl compound and other neonicotinoids well overlapped, and dinotefuran adopts the active conformation more easily than the methoxypropyl compound. © Pesticide Science Society of Japan

Keywords: neonicotinoids, ($\pm$)-tetrahydro-3-furylmethyl, dinotefuran, acetylcholine, structure-activity relationships (SAR), 3D-QSAR.

INTRODUCTION

Neonicotinoid is a major chemical class of insecticide, whose sales have expanded because of its high level of activity and residual control properties. Seven neonicotinoids have been developed, and classified based on structural differences of a hydrogen acceptor site into three sub-classes: chloronicotinyl, thianicotinyl and furanicotinyl (Fig. 1).^{1,2)} The chloronicotinyl and thianicotinyl compounds have pyridine-like moieties, as is the case for the pyridine ring in nicotine (1), and the furanicotinyl compound dinotefuran (2) has a characteristic ($\pm$)-tetrahydro-3-furylmethyl moiety.³⁾

The ($\pm$)-tetrahydro-3-furylmethyl moiety was detected by cyclization of the 3-methoxypropyl moiety which was led from acetylcholine (Fig. 2). These moieties and the pyridine ring are similar in the location of their oxygen and nitrogen atoms (Fig. 1), but the details have not been clarified. In this paper we report the SAR (structure-activity relationships) of substituted (tetrahydro-3-furyl)methyl compounds and the molecular modeling of these derivatives, and discuss the relationship between tetrahydro-3-furan moieties and the pyridine-like moieties.

MATERIALS AND METHODS

1. Instrumental Analysis

Melting points were obtained on a Mettler FP62 melting point apparatus and were uncorrected. ¹H NMR spectra were recorded on a JEOL JNM-LA400 spectrometer using tetramethylsilane as an internal standard. IR spectra were recorded on a JASCO FT/IR-7300 spectrometer. Analytical TLC was performed on silica gel 60 F₂₅₄ (Merck). Spots were detected under UV light or with iodine.

2. Molecular Modeling

All computations were performed using the molecular modeling software package SYBYL, version 6.9 (Tripos Associates, Inc., St. Louis, MO, USA). Energies were calculated with the Merck force field (MMFF94S)⁴⁾ and MMFF94 charges.⁵⁾ A dielectric permittivity of $\epsilon_r = 1$ and a non-cutoff were used. In this computational analysis, the (*R*)-enantiomer of dinotefuran was used because there is no great difference in activity between (*R*) and (*S*) (data not shown).

To obtain conformational databases, each compound was subjected to a systematic search in which all rotatable bonds were allowed to vary in 30 steps (acyclic bonds) and 10 steps (ring closure bonds). The remaining structures were subsequently minimized with an electrostatic term until the rms (root-mean-square) gradient was less than 10^{-3} kcal mol⁻¹ Å⁻¹. Structures that converged to the same

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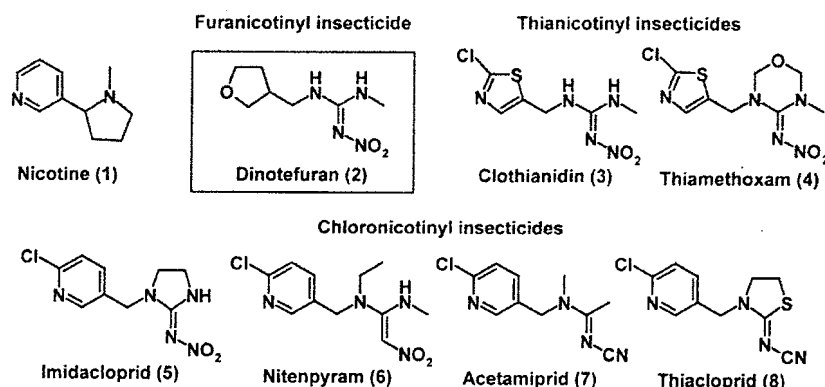


Fig. 1. Nicotine and Neonicotinoids.

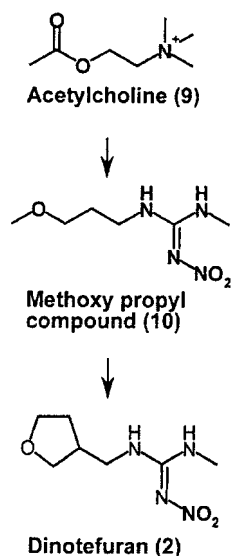


Fig. 2. Discovery of dinotefuran (2).

minimum were omitted on the basis of their energies and by an rms fitting procedure in which all non-hydrogen atoms were superimposed using 0.2 Å as a similarity cutoff. The remaining conformers were stored in conformational databases. Furthermore, the conformations of the nitroimino parts were filtered into one conformation (see Fig. 4), the rest being excluded from the databases.

To obtain pharmacophores among active compounds in a multiconformation structure database, we used the DISCO⁶⁾ (DIStance COmparison) program, an algorithm available in the Sybyl software. The first step of the DISCO search routine consists of identifying all potential pharmacophoric site points in each molecule. The site point assignments include aromatic and aliphatic ring centroids, functional groups with a hydrogen bond donor or acceptor potential, and external site points that represent receptor-associated hydrogen bond acceptors or donors. Secondly, the DISCO method was used to

identify the most probable active conformations.

3. Synthesis

Four types of (±)-tetrahydro-3-furylmethyl compounds, acyclic nitroimino and nitromethylene (xvi), cyclic nitroimino and nitromethylene (xvii) with excellent insecticidal activities were synthesized according to the following methods (Fig. 3): 3- and 4-substituted derivatives (iv and v) were prepared by the reduction of triesters followed by cyclization⁷⁾ of triols (iii), which were obtained by the substitution of esters (ii) with malonates (i), followed by mesylation and amination. Four- and 5-substituted derivatives (vii and viii) were prepared by the same method as iv and v using vi instead of ii, and 5,5-dimethyl derivatives (x and xi) were prepared using ix. The 2-methyl derivative (xiii) was prepared by the reduction of oxime, which was synthesized from xii⁸⁾ and a 2-methoxy derivative (xv) was prepared by the reduction of amide which was synthesized from xiv.⁹⁾ Acyclic and cyclic (tetrahydro-3-furyl)methyl compounds (xvi and xvii) were synthesized according to published procedures,³⁾ using amines (v, viii, xi, xiii and xv) and mesylates (iv, vii and x). Typical procedures are described as follows.

3.1. Typical synthesis of acyclic nitroimino compounds (xvi)

3.1.1. 1-Methylthio-2-nitro-1-[(4-ethyl)tetrahydro-3-furyl]methylamino]ethylene (26)

To a stirred solution of sodium (3.55 g, 154 mmol) in EtOH (100 ml) at room temperature, diethyl malonate (i, 24.6 g, 154 mmol) was added dropwise. The mixture was stirred at 70°C for 30 min, then ethyl 2-bromobutyrate (ii, 30.0 g, 154 mmol) was added dropwise at room temperature. After stirring at 70°C for 5 hr, water (100 ml) was added and extracted with EtOAc (100 ml × 3). The organic layer was washed with brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure to give 44.2 g of crude ethyl 2-[bis(ethoxycarbonyl)methyl]butyrate as an oil, ¹H NMR δ (CDCl₃): 0.93 (3H, t, *J*=7.3 Hz), 1.22–1.31 (9H, m), 1.53–1.68 (2H, m), 3.08 (1H, ddd, *J*=4.4 Hz, *J*=8.0 Hz,

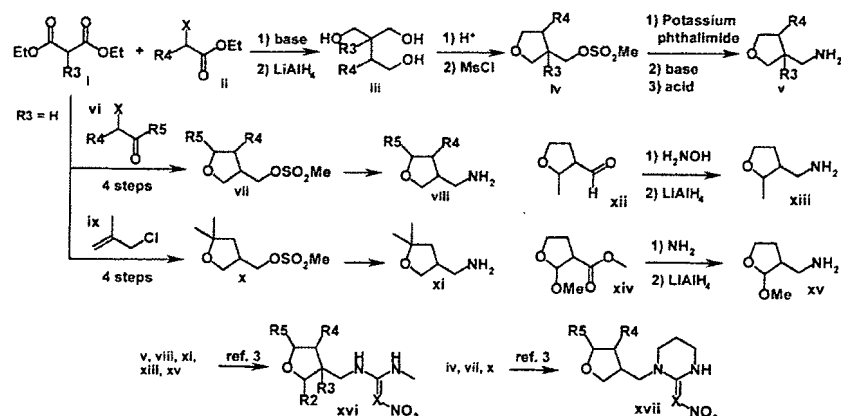


Fig. 3. Synthesis of substituted tetrahydro-3-furylmethyl derivatives xvi and xvii.

$J=11.0$ Hz), 3.75 (1H, d, $J=11.0$ Hz), 4.09–4.28 (6H, m).

To a stirred mixture of LiAlH_4 (12.0 g, 316 mmol) in THF (240 ml) in an ice-cold bath, crude ethyl 2-[(bis(ethoxycarbonylmethyl)methyl]butyrate (44.2 g) was added dropwise. The mixture was refluxed for 9 hr and cooled to room temperature. Water (12.0 g), 3 M NaOH aq. (12.0 g) and water (36.0 g) were added carefully in that order. The resulting solid was filtered off and washed with diethyl ether (200 ml). The combined filtrate was concentrated under reduced pressure to give crude 2-ethyl-3-hydroxymethyl-1,4-butanediol (iii, 19.5 g) as an oil.

A solution of crude 2-ethyl-3-hydroxymethyl-1,4-butanediol (19.5 g) and polyphosphoric acid (10 ml) was stirred at 100°C for 4 hr. Water (15 ml) was added and neutralized with potassium carbonate and extracted with diethyl ether (100 ml $\times$ 2). The organic layer was washed with brine, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The resulting material was purified by silica gel column chromatography (hexane:EtOAc=4:1–1:1) to give 14.5 g (73%, 3 steps) of 3-ethyl-4-(hydroxymethyl)tetrahydrofuran as an oil. ^1H NMR δ (CDCl_3): 0.93 (3H, t, $J=7.3$ Hz), 1.33–1.61 (2H, m), 1.77–1.86 (1H, m), 2.00–2.10 (1H, m), 3.39 (1H, dd, $J=7.3$ Hz, $J=8.8$ Hz), 3.56 (1H, dd, $J=7.3$ Hz, $J=10.3$ Hz), 3.67–3.73 (2H, m), 3.89 (1H, dd, $J=7.3$ Hz, $J=8.8$ Hz), 3.99 (1H, t, $J=7.3$ Hz).

To a stirred solution of 3-ethyl-4-(hydroxymethyl)tetrahydrofuran (11.5 g, 88.3 mmol) and methanesulfonylchloride (10.1 g, 88.5 mmol) in THF (80 ml) at room temperature, was added triethylamine (9.10 g, 89.9 mmol) and the mixture was further stirred for 6 hr. The mixture was quenched with water (50 ml) and extracted with EtOAc (70 ml $\times$ 2). The organic layer was dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The resulting material was purified by silica gel column chromatography (hexane:EtOAc=8:1–1:1) to give 7.60 g (41%) of 3-ethyl-4-(methanesulfonyloxymethyl)tetrahydrofuran as an oil.

A solution of 3-ethyl-4-(methanesulfonyloxymethyl)tetrahydrofuran (2.93 g, 14.1 mmol) and potassium phthalimide

(2.70 g, 14.6 mmol) in DMF (15 ml) was stirred at 60°C for 7 hr. The mixture was quenched with water (50 ml) and extracted with EtOAc (100 ml). The organic layer was washed with water (50 ml $\times$ 2), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure, to give 3.00 g (82%) of *N*-[[(4-ethyl)tetrahydrofuran-3-yl]methyl]phthalimide as an oil.

A solution of *N*-[[(4-ethyl)tetrahydrofuran-3-yl]methyl]phthalimide (2.98 g, 11.5 mmol) and 25% NaOH aq. (2.0 g) in water (8 ml) was stirred at 70°C for 3 hr and the solution added to 10% HCl aq. (8 ml) at 70°C and stirred for 4 hr. Toluene (15 ml) was added and stirred for 30 min at 70°C , then the water layer and solid were alkalinized with 50% NaOH aq. The solution was extracted with chloroform (80 ml $\times$ 3) dried over anhydrous magnesium sulfate, and concentrated under reduced pressure, to give 1.05 g of crude 3-aminomethyl-4-ethyltetrahydrofuran as an oil.

A solution of crude 3-aminomethyl-4-ethyltetrahydrofuran (1.05 g) and 1,1-bis(methylthio)-2-nitroethylene (1.20 g, 7.26 mmol) in acetonitrile (15 ml) was stirred at 70°C for 5 hr. The mixture was concentrated to dryness under reduced pressure. The resulting material was purified by silica gel column chromatography (hexane:EtOAc=1:1) to give 1.27 g (45%, 2 steps) of 1-methylthio-2-nitro-1-[(4-ethyl)tetrahydro-3-furyl]methylamino]ethylene as a yellow solid. ^1H NMR δ (CDCl_3): 0.94 (3H, t, $J=7.3$ Hz), 1.37–1.60 (2H, m), 1.81–1.92 (1H, m), 2.15–2.27 (1H, m), 2.45 (3H, s), 3.37–3.48 (3H, m), 3.63 (1H, dd, $J=5.1$ Hz, $J=9.5$ Hz), 3.89 (1H, dd, $J=7.3$ Hz, $J=9.5$ Hz), 4.05–4.16 (1H, m), 6.58 (1H, s), 10.6 (1H, br). IR (KBr) cm^{-1} : 3410, 1561.

A solution of 1-methylthio-2-nitro-1-[(4-ethyl)tetrahydro-3-furyl]methylamino]ethylene (0.37 g, 1.5 mmol) in 40% MeNH₂ (in MeOH, 1.0 g) was stirred at room temperature for 1 hr. The mixture was purified by silica gel column chromatography (EtOAc:MeOH=5:1) to give 0.27 g (79%) of 1-methylamino-2-nitro-1-[(4-ethyl)tetrahydro-3-furyl]methylamino]ethylene (26) as a colorless solid, Mp $121\text{--}124^\circ\text{C}$. ^1H

NMR δ (CDCl₃): 0.94 (3H, t, $J=7.3$ Hz), 1.40–1.65 (2H, m), 1.82–1.92 (1H, br), 2.07–2.25 (1H, br), 2.82–2.95 (3H, br), 3.10–3.45 (4H, br), 3.70–3.85 (2H, br), 5.50–5.80 (1H, br), 6.58 (1H, s), 10.2–10.4 (1H, br). IR (KBr) cm⁻¹: 3256, 1566.

3.1.2. 1-Methyl-2-nitro-3-[(2-methyl)tetrahydro-3-furylmethyl]guanidine (14)

Oil. ¹H NMR δ (CDCl₃): 1.26 (3H, d, $J=6.6$ Hz), 1.66–1.76 (2H, m), 2.09–2.27 (2H, m), 2.97 (3H, d, $J=5.1$ Hz), 3.35–3.39 (3H, m), 3.72–3.94 (3H, m). IR (neat) cm⁻¹: 3306, 1618.

3.1.3. 1-Methyl-2-nitro-3-[(2-methoxy)tetrahydro-3-furylmethyl]guanidine (15)

Oil. ¹H NMR δ (CDCl₃): 1.60–1.75 (1H, m), 2.20–2.33 (1H, m), 2.42–2.57 (1H, m), 2.95–2.97 (3H, m), 3.28–3.40 (2H, m), 3.36 (2/3×3H, s), 3.39 (1/3×3H, s), 3.88–4.12 (2H, m), 4.81 (2/3×1H, d, $J=2.2$ Hz), 5.10 (1/3×1H, d, $J=2.2$ Hz).

3.1.4. 1-Methyl-2-nitro-3-[(3-methyl)tetrahydro-3-furylmethyl]guanidine (16)

Oil. ¹H NMR δ (CDCl₃): 1.19 (3H, s), 1.78–1.92 (1H, m), 2.93 (3H, d, $J=5.1$ Hz), 3.15–3.40 (3H, m), 3.77–3.91 (2H, m), 4.04 (1H, dt, $J=5.1$ Hz, $J=8.8$ Hz). IR (neat) cm⁻¹: 3340, 1645.

3.1.5. 1-Methyl-2-nitro-3-[(4-methyl)tetrahydro-3-furylmethyl]guanidine (17)

Oil. ¹H NMR δ (CDCl₃): 1.05 (1/10×3H, d, $J=6.6$ Hz), 1.11 (9/10×3H, d, $J=6.6$ Hz), 1.86 (2H, br), 2.01–2.14 (2H, m), 2.95–2.98 (3H, m), 3.28–3.38 (3H, m), 3.67 (9/10×1H, dd, $J=4.4$ Hz, $J=8.8$ Hz), 3.74 (1/10×1H, dd, $J=4.4$ Hz, $J=8.8$ Hz), 3.90–4.07 (2H, t, m). IR (neat) cm⁻¹: 3304, 1618.

3.1.6. 1-Methyl-2-nitro-3-[(4-ethyl)tetrahydro-3-furylmethyl]guanidine (18)

Oil. ¹H NMR δ (CDCl₃): 0.94 (3H, t, $J=7.3$ Hz), 1.38–1.59 (2H, m), 1.82–1.95 (1H, m), 2.15–2.27 (1H, m), 2.95 (3H, d, $J=5.1$ Hz), 3.30–3.43 (3H, m), 3.69 (1H, dd, $J=3.7$ Hz, $J=9.5$ Hz), 3.81 (1H, dd, $J=6.6$ Hz, $J=9.5$ Hz), 4.09 (3H, t, $J=8.1$ Hz). IR (neat) cm⁻¹: 3305, 1618.

3.1.7. 1-Methyl-2-nitro-3-[(3,4-dimethyl)tetrahydro-3-furylmethyl]guanidine (19)

Oil. ¹H NMR δ (CDCl₃): 0.96–1.00 (3H, m), 1.02 (3/5×3H, s), 1.027 (2/5×3H, s), 2.04–2.22 (1H, m), 2.91–2.96 (3H, m), 3.05–3.21 (1H, m), 3.32–3.58 (3H, m), 4.71–4.90 (1H, m), 4.11–4.24 (1H, m). IR (neat) cm⁻¹: 3339, 1643.

3.1.8. 1-Methyl-2-nitro-3-[(5-methyl)tetrahydro-3-furylmethyl]guanidine (20)

Oil. ¹H NMR δ (CDCl₃): 1.22 (1/2×3H, d, $J=6.6$ Hz), 1.29 (1/2×3H, d, $J=6.6$ Hz), 1.54–1.88 (2H, m), 2.05 (3H, s), 2.88–2.96 (2H, m), 3.33–4.20 (4H, m). IR (neat) cm⁻¹: 3313, 1619.

3.1.9. 1-Methyl-2-nitro-3-[(5-phenyl)tetrahydro-3-furylmethyl]guanidine (21)

Oil. ¹H NMR δ (CDCl₃): 1.52–1.63 (1/2×1H, m), 1.99–2.22 (1/2×1H+1H, m), 2.51–2.61 (1/2×1H, m), 2.67–2.80 (1/2×1H, m), 2.84 (1/2×3H, d, $J=5.1$ Hz), 2.95 (1/2×3H, d, $J=5.1$ Hz), 3.41 (2H, d, $J=5.1$ Hz), 3.70–3.78 (1/2×1H, m),

3.86–4.01 (1H, m), 4.08–4.23 (1/2×1H, m), 4.84–4.89 (1/2×1H, m), 5.01–5.07 (1/2×1H, m), 7.23–7.36 (5H, m). IR (neat) cm⁻¹: 3301, 1617.

3.1.10. 1-Methyl-2-nitro-3-[(5,5-dimethyl)tetrahydro-3-furylmethyl]guanidine (22)

Oil. ¹H NMR δ (CDCl₃): 1.22 (3H, s), 1.33 (3H, s), 1.43 (1H, dd, $J=7.3$ Hz, $J=12.5$ Hz), 2.01 (1H, dd, $J=7.3$ Hz, $J=12.5$ Hz), 2.68 (1H, septet, $J=7.3$ Hz), 2.97 (3H, d, $J=4.4$ Hz), 3.35 (1H, t, $J=4.4$ Hz), 3.62 (1H, dd, $J=4.4$ Hz, $J=8.8$ Hz), 3.95 (1H, dd, $J=7.3$ Hz, $J=8.8$ Hz). IR (neat) cm⁻¹: 3305, 1616.

3.1.11. 1-Methylamino-2-nitro-1-[(2-methyl)tetrahydro-3-furyl]methyldiamino]ethylene (23)

Mp 127–128°C. ¹H NMR δ (CDCl₃): 1.26–1.33 (3H, br), 1.64–1.76 (2H, br), 2.14–2.30 (2H, br), 2.88–2.96 (3H, br), 3.20–3.26 (2H, m), 3.71–3.98 (3H, m), 6.59 (1H, s), 10.3 (1H, br). IR (KBr) cm⁻¹: 3277, 1626.

3.1.12. 1-Methylamino-2-nitro-1-[(2-methoxy)tetrahydro-3-furyl]methyldiamino]ethylene (24)

Oil. ¹H NMR δ (DMSO): 1.45–1.60 (1H, br), 1.90–2.10 (1H, br), 2.70–2.80 (1H, br), 3.21 (3H, s), 3.34 (3H, s), 3.30–3.40 (2H, br), 3.75 (1H, q, $J=8.1$ Hz), 3.90 (1H, br), 4.83 (1H, br), 6.46 (1H, br), 7.25–7.73 (1H, br), 9.96–10.1 (1H, br).

3.1.13. 1-Methylamino-2-nitro-1-[(4-methyl)tetrahydro-3-furyl]methyldiamino]ethylene (25)

Mp 127–129°C. ¹H NMR δ (CDCl₃): 0.95 (1/10×3H, d, $J=6.6$ Hz), 1.00 (9/10×3H, d, $J=6.6$ Hz), 1.90–2.05 (2H, m), 2.65–2.85 (2H, br), 3.15–3.45 (4H, br), 3.75–3.90 (4H, m), 6.47 (1H, br), 10.1 (1H, br). IR (KBr) cm⁻¹: 3273, 1628.

3.1.14. 1-Methylamino-2-nitro-1-[(5-methyl)tetrahydro-3-furyl]methyldiamino]ethylene (27)

Mp 114–120°C. ¹H NMR δ (KBr): 1.12–1.30 (4H, m), 1.68–1.88 (1H, m), 2.19–2.29 (1H, m), 2.64 (1H, br), 2.87 (1/2×3H, d, $J=5.1$ Hz), 2.99 (1/2×3H, d, $J=5.1$ Hz), 3.20–3.45 (2H, br), 3.72–4.10 (3H, br), 6.60 (1H, s), 10.2 (1H, br).

3.1.15. 1-Methylamino-2-nitro-1-[(5,5-dimethyl)tetrahydro-3-furyl]methyldiamino]ethylene (28)

Mp 132–133°C. ¹H NMR δ (CDCl₃): 1.22 (3H, s), 1.33 (3H, s), 1.44–1.54 (1H, m), 2.02 (1H, dd, $J=8.1$ Hz, $J=12.5$ Hz), 2.69 (1H, septet, $J=7.3$ Hz), 2.87 (1/2×3H, d, $J=4.4$ Hz), 3.00 (1/2×3H, d, $J=4.4$ Hz), 3.20–3.36 (2H, m), 3.56–3.62 (2H, m), 3.94–4.00 (2H, m), 6.32 (1/2×1H, br), 6.60 (1H, s), 6.61 (1/2×1H, br), 10.3 (1H, br). IR (KBr) cm⁻¹: 3192, 1616.

3.1.16. 2-Nitromethylene-1-[(4-methyl)tetrahydro-3-furyl]methyl]hexahydropyrimidine (29)

Mp 94–97°C. ¹H NMR δ (CDCl₃): 1.01 (1/10×3H, d, $J=6.6$ Hz), 1.10 (9/10×3H, d, $J=6.6$ Hz), 1.62–2.10 (3H, m), 2.14–2.27 (1H, m), 3.09 (1H, dd, $J=8.8$ Hz, $J=14.7$ Hz), 3.30–3.48 (6H, m), 3.56 (1H, dd, $J=5.9$ Hz, $J=8.8$ Hz), 3.90 (1H, dd, $J=6.6$ Hz, $J=8.8$ Hz), 4.05 (1H, dd, $J=7.3$ Hz, $J=8.8$ Hz), 6.63 (1/10×1H, s), 6.65 (9/10×1H, s), 10.1 (1H, br). IR (KBr) cm⁻¹: 3490, 1585.

3.1.17. 2-Nitromethylene-1-[(4-ethyl)tetrahydro-3-furyl]methyl]hexahydropyrimidine (30)

Oil. ^1H NMR δ (CDCl_3): 0.93 (3H, t, $J=7.3$ Hz), 1.33–1.59 (2H, m), 1.71–1.82 (1H, m), 2.01–2.09 (2H, m), 2.21–2.32 (1H, m), 3.10 (1H, dd, $J=8.8$ Hz, $J=14.7$ Hz), 3.31 (1H, dd, $J=6.6$ Hz, $J=14.7$ Hz), 3.35–3.48 (4H, m), 3.56 (1H, dd, $J=5.1$ Hz, $J=8.8$ Hz), 3.72 (1H, q, $J=7.3$ Hz), 3.81 (1H, dd, $J=7.3$ Hz, $J=8.8$ Hz), 4.06 (1H, dd, $J=7.3$ Hz, $J=8.8$ Hz), 6.65 (1H, s), 9.75 (1H, br). IR (neat) cm^{-1} : 3428, 1589.

3.1.18. 2-Nitromethylene-1-[(5-methyl)tetrahydro-3-furyl]methyl]hexahydropyrimidine (31)

Mp 92–93°C. ^1H NMR δ (CDCl_3): 1.23 (1/2×3H, d, $J=6.6$ Hz), 1.29 (1/2×3H, d, $J=6.6$ Hz), 1.60–1.81 (2H, m), 2.01–2.09 (2H, m), 2.63–2.76 (1H, m), 3.08–3.35 (2H, m), 3.40–3.48 (5H, m), 3.59–3.63 (1/3×1H, m), 3.76–3.82 (1/2×1H, m), 3.89–4.01 (1/2×1H, m), 4.09–4.21 (2/3×1H, m), 6.65 (1H, s), 10.3 (1H, br). IR (KBr) cm^{-1} : 3448, 1589.

3.1.19. 2-Nitromethylene-1-[(5,5-dimethyl)tetrahydro-3-furyl]methyl]hexahydropyrimidine (32)

Mp 119–121°C. ^1H NMR δ (CDCl_3): 1.21 (3H, s), 1.32 (3H, s), 1.43 (1H, dd, $J=8.1$ Hz, $J=12.4$ Hz), 2.04 (1H, dd, $J=8.1$ Hz, $J=12.5$ Hz), 2.10 (2H, quintet, $J=5.9$ Hz), 2.73 (1H, septet, $J=8.1$ Hz), 3.21 (1H, dd, $J=8.1$ Hz, $J=14.7$ Hz), 3.32 (1H, dd, $J=6.6$ Hz, $J=14.7$ Hz), 3.42–3.48 (4H, m), 3.52 (1H, dd, $J=6.6$ Hz, $J=8.8$ Hz), 3.98 (1H, dd, $J=7.3$ Hz, $J=8.8$ Hz), 6.62 (1H, s), 9.88 (1H, br). IR (KBr) cm^{-1} : 1585, 1431.

3.1.20. 2-Nitroimino-1-[(4-methyl)tetrahydro-3-furyl]methyl]hexahydropyrimidine (33)

Oil. ^1H NMR δ (CDCl_3): 1.01 (1/10×3H, d, $J=6.6$ Hz), 1.07 (9/10×3H, d, $J=6.6$ Hz), 1.67–2.10 (3H, m), 2.14–2.27 (1H, m), 3.31–3.53 (6H, m), 3.58 (1H, dd, $J=6.6$ Hz, $J=8.8$ Hz), 3.68 (1H, dd, $J=6.6$ Hz, $J=13.7$ Hz), 3.92 (1H, dd, $J=7.3$ Hz, $J=8.8$ Hz), 4.02 (1H, t, $J=7.3$ Hz), 9.78 (1H, br). IR (neat) cm^{-1} : 3276, 1735.

3.1.21. 2-Nitroimino-1-[(5-methyl)tetrahydro-3-furyl]methyl]hexahydropyrimidine (34)

Oil. ^1H NMR δ (CDCl_3): 1.23 (1/2×3H, d, $J=6.6$ Hz), 1.27 (1/2×3H, d, $J=6.6$ Hz), 1.55–1.86 (2H, m), 2.01–2.15 (2H, m), 2.69–2.86 (1H, m), 3.32–3.50 (5H, m), 3.57–3.84 (2H, m), 3.92–4.00 (1H, m), 4.08–4.18 (1H, m), 9.78 (1H, br). IR (neat) cm^{-1} : 3275, 1592.

3.1.22. 2-Nitroimino-1-[(5,5-dimethyl)tetrahydro-3-furyl]methyl]hexahydropyrimidine (35)

Oil. ^1H NMR δ (CDCl_3): 1.21 (3H, s), 1.31 (3H, s), 1.47 (1H, dd, $J=8.8$ Hz, $J=12.5$ Hz), 1.91 (1H, dd, $J=8.8$ Hz, $J=12.5$ Hz), 2.01–2.09 (2H, m), 2.71–2.88 (1H, m), 3.40–3.67 (7H, m), 3.94 (1H, dd, $J=7.3$ Hz, $J=8.8$ Hz), 9.78 (1H, br). IR (neat) cm^{-1} : 3285, 1592.

4. Biological Tests

4.1. Insects

Biological tests were conducted against the small brown planthopper, *Laodelphax striatellus* Fallén, and the green rice

leafhopper, *Nephotettix cincticeps*.

All tests were replicated twice and done at $25(\pm 2)^\circ\text{C}$. at $65(\pm 5)\%$ RH and under a 16:8 h light:dark photoperiod.

4.2. Contact/feeding activity in *L. striatellus* and *N. cincticeps*

A bundle of rice seedlings (about third-leaf stage) was sprayed with water+acetone (4+1 by volume) containing the test compound at a concentration of 1, 10, 100 or 1000 ppm. After drying, the treated seedlings were covered with a metal gauze cylinder, and 10 one- to three-day-old male adults of *L. striatellus* or *N. cincticeps* were released into the cylinder. Mortality was checked 3 days later.

Insecticidal activity was graded as follows: 0: $\text{LC}_{70} > 1000$ ppm, 1: 1000–100 ppm, 2: 100–10 ppm, 3: 10–1 ppm, 4: 1–0.1 ppm.

RESULTS AND DISCUSSION

1. Conformational Analysis of Dinotefuran (2), Clothianidin (3), Imidacloprid (5) and a 3-Methoxypropyl Compound (10)

When the 3-methoxypropyl moiety was cyclized to the ($\pm$)-(tetrahydro-3-furyl)methyl moiety, dinotefuran (2) exhibited more than a 100-fold higher level of activity than the 3-methoxypropyl compound (10) against *N. cincticeps*. We assumed that dinotefuran and other neonicotinoids have a common 3D-pharmacophore for binding to nicotinic acetylcholine receptors because of their structural similarity and mode of action,¹⁰ and that it would be difficult for 10 to have the same conformation because of its weak activity. In order to test this hypothesis, a conformational analysis of neonicotinoids (dinotefuran (2), clothianidin (3), imidacloprid (5)) and the 3-methoxypropyl compound (10) was carried out.

First of all, we generated individual conformer databases according to the procedure described in the methods. The conformational databases were then entered into the DISCO program to perform the superimposition of the four compounds. The different superimposition models proposed by DISCO were grouped into three types that showed a good overlay of the compounds (Fig. 4). Superposition A, B and C shown in Fig. 2 correspond to the active conformation models proposed by Okazawa *et al.*^{11,12} and Samaritoni *et al.*¹³ respectively. In all superposition models, the hypothetical receptor-associated hydrogen bond acceptor sites generated from the tetrahydro-3-furan, pyridine and thiazole rings are located in the same place. Thus, the oxygen atom in the tetrahydro-3-furan group of dinotefuran (2) can function as the hydrogen acceptor site in place of the nitrogen atoms in the pyridine or thiazole rings.

At the end of the conformational search, we obtained 16 and 78 energy-minimized conformers for dinotefuran (2) and compound 10 with energy ranges (the energy difference between the highest and the lowest energy conformer) of 4.5 and 8.1 kcal/mol, respectively. Three conformations out of 16 have the receptor-associated hydrogen bond acceptor within

2 Å from that of the active conformer when the imidazolidine parts are superposed onto superposition A. Twenty-four percent of all energy-minimized conformations took superposition A on the basis of the Boltzmann probability distribution

at room temperature (300 kelvin). On the other hand, six conformations among 78 can take the conformation (superposition A) in 10, but this was only 4.5%.

The straight-chain ether is so flexible that 10 has to lose

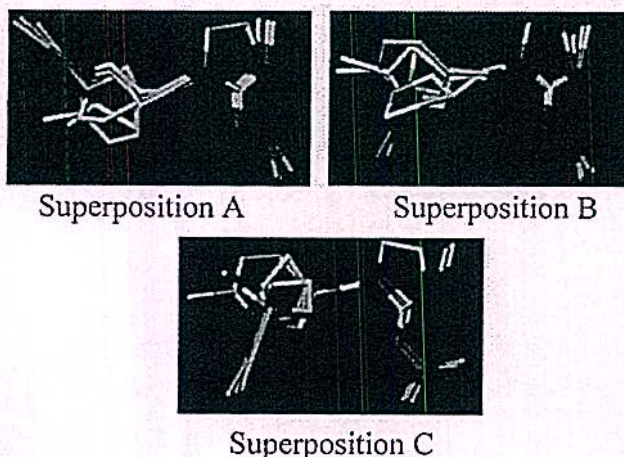


Fig. 4. Molecular superpositions of known neonicotinoids and the 3-methoxypropyl compound (10) using a hydrogen-devoid backbone-type representation. The carbons of dinotefuran (2), clothianidin (3), imidacloprid (5) and 10 are shown in white, green, orange and cyan, respectively. The oxygen, nitrogen and sulfur are shown in red, blue and yellow, respectively. The hydrogen acceptor site defined in DISCO is also shown in magenta. Hydrogen atoms are not shown.

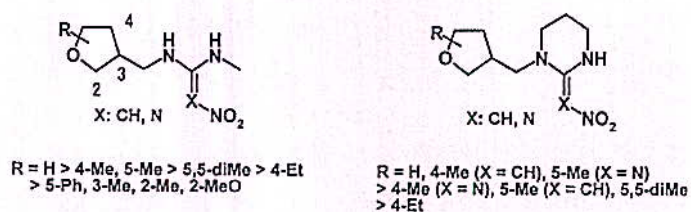


Fig. 5. SARs of the acyclic and cyclic compounds.

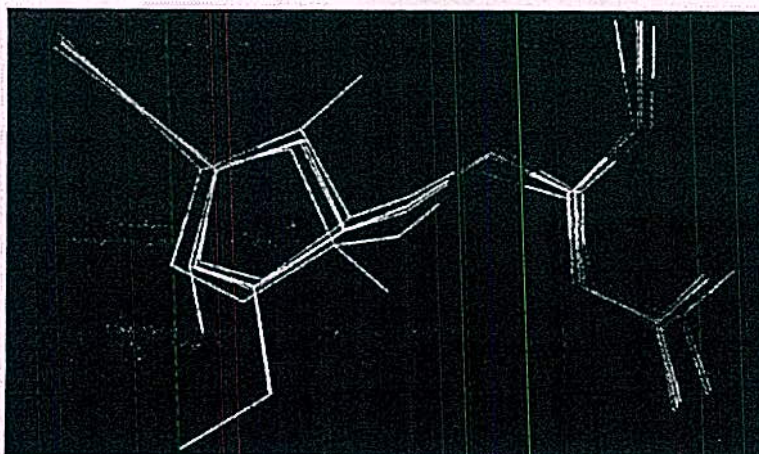


Fig. 6. Molecular superpositions of the acyclic nitroimino compounds with the Superposition A conformation. Carbon, oxygen and nitrogen are shown in white, red and blue, respectively. Hydrogen atoms are not shown. Dinotefuran is shown in green.

more entropy upon binding. On the other hand, cyclization of the ether group in dinotefuran (2) restricts the conformational degree of freedom and then reduces the entropy loss upon binding. We speculate that the reduced loss of conformational entropy of 2 upon binding (structural rigidity) contributes considerably to the activity as compared with 10. However, 2 was over 100-fold more active than 10 (data not shown). These results suggest that the weak activity of 10 would be caused by the interaction with the receptor.

2. SAR for the Tetrahydro-3-furan Part of Dinotefuran . Four types of insecticidal activities; acyclic nitroimino compounds (2 and 14–22), acyclic nitromethylene compounds (11 and 23–28), cyclic nitroimino compounds (13 and 33–35) and cyclic nitromethylene compounds (12 and 29–32), are shown in Tables 1–3. In general, all four types have a similar SAR for the tetrahydro-3-furan part (Fig. 5). The 2- or 3-substitutions on tetrahydro-3-furan were detrimental to the activity, and the 4- and 5-substitutions reduced the activity to some extent but there is a size limitation. The details are as follows.

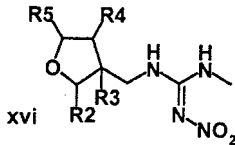
Unsubstituted compounds (2 and 11–13) showed the highest level of activity for each type. The 2-Me or 3-Me substituted compounds (14–16, 19 and 23–24) lost activity. In regard to the 4- and/or 5-substituted compounds, various levels of activity were observed. As for acyclic nitroimino compounds, 4-Me and 5-Me substituted compounds (17 and 20) were 100-fold less active against *L. striatellus* and 10-fold less active against *N. cincticeps* than 2. Increasing the size of

the substituent from a methyl to ethyl, phenyl or dimethyl lowered the level of activity (18, 21 and 22). Acyclic nitromethylene compounds showed the same SAR but the activity of cyclic compounds was not as weak as that of acyclic types. For example, the 4-Me-substituted nitromethylene compound (29) and the 5-Me-substituted nitroimino compound (34) showed the same level of activity as each unsubstituted compounds (12 and 13), and the other Me-substituted compounds did not exhibit a drop in activity of more than 10-fold (31–33 and 35) either.

3. Structural Analysis of Substituted (Tetrahydro-3-furyl)methyl Compounds

The conformational search was also performed with acyclic nitroimino compounds (Fig. 6; 14, 16–18 and 20). The substituent on the tetrahydrofuran ring, which has the trans con-

Table 1. Insecticidal activities of acyclic nitroimino compounds xvi



xvi

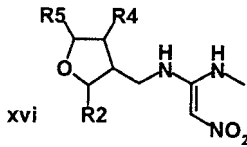
No.	R1–R5	LS	NC
2	H	4	4
14	2-Me ^a	0	0
15	2-MeO ^b	0	0
16	3-Me	0	0
17	4-Me ^c	2	3
18	4-Et ^d	0	1
19	3-Me, 4-Me ^e	0	0
20	5-Me ^f	2	3
21	5-phenyl ^g	0	0
22	5-Me, 5-Me	1	3

LS: *Laodelphax striatellus*.

NC: *Nephotettix cincticeps*.

^{a–g}Diastereo mixtures (determined by ¹H NMR. ^atrans, ^b(2 : 1), ^c(9 : 1), ^dtrans, ^e(3 : 2), ^f(1 : 1), ^g(1 : 1)).

Table 2. Insecticidal activities of acyclic nitromethylene compounds xvi

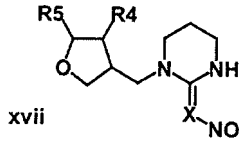


xvi

No.	R2–R5	LS	NC
11	H	4	4
23	2-Me ^a	0	0
24	2-MeO ^b	0	0
25	4-Me ^c	3	3
26	4-Et ^d	0	1
27	5-Me ^f	2	3
28	5-Me, 5-Me	2	3

Abbreviations, see Table 1.

Table 3. Insecticidal activities of cyclic compounds xvii



xvii

No.	X	R4, R5	LS	NC
12	CH	H	3	4
29	CH	4-Me ^c	3	4
30	CH	4-Et ^d	1	0
31	CH	5-Me ^f	3	3
32	CH	5-Me, 5-Me	3	3
13	N	H	3	4
33	N	4-Me ^c	2	4
34	N	5-Me ^f	3	4
35	N	5-Me, 5-Me	2	3

Abbreviations, see Table 1.

figuration, was used. The conformational databases were then entered into the DISCO program to perform the superimposition of all compounds with dinotefuran, clothianidin and imidacloprid. All compounds could satisfy the conditions of superpositions A, B and C with the energy-minimized conformations. A typical example of superposition A is shown in Fig. 5. Their potential energies *in vacuo* are relatively low (within 3 kcal mol⁻¹ from the lowest energy conformation). Thus, the weak activity of compounds **14** and **16** might be caused not by the difficulty in adopting the active conformation but by other factors, such as the interaction with the receptor and so on.

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United States Patent [19]
Twydell et al.

[11] Patent Number: 4,833,158
[45] Date of Patent: May 23, 1989

- [54] METHOD OF COMBATING TERMITES
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- [21] Appl. No.: 133,983
- [22] Filed: Dec. 17, 1987
- [30] Foreign Application Priority Data
Jan. 15, 1987 [GB] United Kingdom 8700838
- [51] Int. Cl.⁴ A01N 37/18; A01N 47/28
- [52] U.S. Cl. 514/616; 514/594; 514/936; 514/946
- [58] Field of Search 514/594, 936, 946, 616
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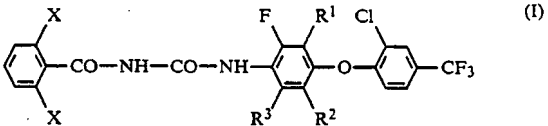
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Primary Examiner—Douglas W. Robinson
Assistant Examiner—Zohreh A. Fay

[57] ABSTRACT

This invention provides a method of combating termites at a locus, which comprises treating the locus e.g. soil or timber with an acyl urea of the formula



wherein one substituent X is a halogen atom or a methyl group and the other is a halogen atom, a hydrogen atom or a methyl group, and each of R¹, R² and R³ independently represents a hydrogen or fluorine atom; the use of acyl ureas of formula I for combating termites; and compositions for protecting timber against termite attack.

7 Claims, No Drawings

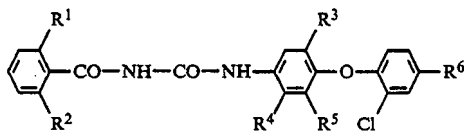
METHOD OF COMBATING TERMITES

This invention relates to a method of combating termites, to the use of certain acyl ureas for combating termites, to compositions for protecting timber against termite attack and to timber protected against termite attack.

Termites are insects of the order Isoptera, which can cause considerable structural damage to buildings in warmer climates, being present between latitude 42° N and 42° S. This damage may be prevented, or minimised, by the use of insecticidal compounds active against termites. Such compounds are conventionally applied either to the building or its component members, e.g. by treatment of timber components before incorporation into the building, or to soil area surrounding the building.

The majority of commercially available insecticides do not have the combination of biological and physico-chemical properties necessary for effective termite control (e.g. activity against termites combined with long-term persistence), but the chlorinated hydrocarbon aldrin has proved effective. However, increased regulatory controls on chlorinated hydrocarbons have created a need for a termiticide which combines the necessary activity and persistence with a low mammalian toxicity.

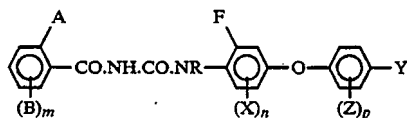
UK Pat. No. 1,460,419 discloses acyl ureas of the formula



in which R¹ is fluorine, chlorine, bromine or methyl, R² is hydrogen, fluorine or chlorine, R³ is hydrogen or chlorine, R⁴ is hydrogen, chlorine or methyl, R⁵ is hydrogen or chlorine, and R⁶ is nitro or trifluoromethyl, and their use as insecticides.

Although the actual insecticide test examples in UK Pat. No. 1,460,419 are limited to the test species diamond-back moth (*Plutella maculipennis*), owl moth (*Laphygma exigua*) and mustard beetle (*Phaedon cochleariae*), the introductory part of the description contains a long list of insect pests to which it is stated that the above compounds may be applied and this list includes "termites such as the eastern subterranean termite (*Reticulitermes flavipes*)".

European Patent Application Publication No. 161019 (EP-A-161019) (Applicant's ref. K 1955) discloses acyl ureas of formula

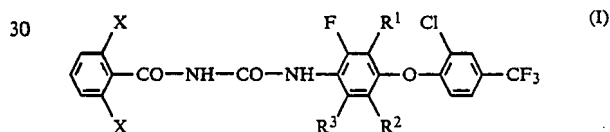


in which each of A and B independently represents a halogen atom or an alkyl group; m is 0 or 1; R represents a hydrogen atom or a group —S.CO₂R¹, —S.SO₂R¹ or —S.NR²R³, in which R¹ represents an optionally substituted alkyl or aryl group; R² represents an optionally substituted alkyl or aryl group; and R³ represents an optionally substituted alkyl or aryl group, or a group of formula —CO₂R⁴, —SO₂R⁴, —COR⁴, —CO-

CO₂R⁴, —CO.NR⁵R⁶ or —SO₂NR⁵R⁶, in which R⁴ represents an optionally substituted alkyl or aryl group, and each of R⁵ and R⁶ independently represents an optionally substituted alkyl or aryl group; or R² and R³ together or R⁵ and R⁶ together represent an optionally substituted alkylene group; in each case, the optional substituents for an alkyl or alkylene group being selected from halogen, alkoxy, alkoxycarbonyl, haloalkoxycarbonyl, alkylcarbonyl, haloalkylcarbonyl, alkylsulphonyl and haloalkylsulphonyl, and the optional substituents for an aryl group being selected from these substituents and also alkyl, haloalkyl, cyano and nitro; X represents a halogen atom or a cyano, nitro, alkyl or haloalkyl group; each of Y and Z independently represents a halogen atom or a cyano, nitro or haloalkyl group; n is 0, 1, 2 or 3; and p is 0, 1 or 2. These acyl ureas are described as having pesticidal, especially insecticidal and acaricidal activity, and test examples demonstrate activity against the insects *Spodoptera littoralis* and *Aedes aegypti* and the mite *Tetranychus urticae*. There is no mention in EP-A-161019 of termites per se.

It has now been found that certain acyl ureas, within the class defined in EP-A-161019, are surprisingly effective termiticides.

Accordingly the present invention provides a method of combating termites at a locus, which comprises treating the locus with an acyl urea of the formula



wherein one substituent X is a halogen atom or a methyl group and the other is a halogen atom, a hydrogen atom or a methyl group, and each of R¹, R² and R³ independently represents a hydrogen or fluorine atom.

Preferably one substituent X is a halogen atom and the other is a hydrogen or halogen atom. Preferably the or each halogen atom is a fluorine or chlorine atom. Advantageously both substituents X are fluorine atoms.

Preferably each of R¹, R² and R³ represents a hydrogen atom.

The acyl urea of formula I wherein both substituents X are fluorine atoms and each of R¹, R² and R³ represents a hydrogen atom is the compound flufenoxuron.

The acyl ureas of formula I may be prepared by the processes described in EP-A-161019.

In order to facilitate the application of the acyl urea to the desired locus, the compound is normally formulated with a carrier and/or a surface-active agent.

A carrier in the present context is any material with which the active ingredient is formulated to facilitate application to the locus to be treated, or to facilitate storage, transport or handling. A carrier may be a solid or a liquid, including a material which is normally gaseous but which has been compressed to form a liquid, and any of the carriers normally used in formulating insecticidal compositions may be used. Preferably compositions according to the invention contain 0.5 to 95% by weight of active ingredient, though proportions as low as 0.001% may be useful in some circumstances.

Suitable solid carriers include natural and synthetic clays and silicates, for example natural silicas such as diatomaceous earths; magnesium silicates, for example talcs; magnesium aluminium silicates, for example at-

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tapulgites and vermiculites; aluminium silicates, for example kaolinites, montmorillonites and micas; calcium carbonate; calcium sulphate; ammonium sulphate; synthetic hydrated silicon oxides and synthetic calcium or aluminium silicates; elements, for example carbon and sulphur; natural and synthetic resins, for example coumarone resins, polyvinyl chloride, and styrene polymers and copolymers; solid polychlorophenols; bitumen; waxes; and solid fertilisers, for example superphosphates.

Suitable liquid carriers include water; alcohols, for example isopropanol and glycols; ketones, for example acetone, methyl ethyl ketone, methyl isobutyl ketone, isophorone and cyclohexanone; ethers; aromatic or araliphatic hydrocarbons, for example benzene, toluene and xylene; petroleum fractions, for example kerosine and light mineral oils; chlorinated hydrocarbons, for example carbon tetrachloride, perchloroethylene and trichloroethane. Polar organic liquids are particularly suitable, such as dimethyl formamide, dimethyl acetamide, dimethyl sulphoxide and N-methylpyrrolidone. Mixtures of different liquids are often suitable, for example a mixture of isophorone or "Shellsol K" (trade mark) with a polar organic solvent, such as N-methylpyrrolidone.

Pesticidal compositions are often formulated and transported in a concentrated form which is subsequently diluted by the user before application. The presence of small amounts of a carrier which is a surface-active agent facilitates this process of dilution. Thus preferably at least one carrier in such a composition is a surface-active agent. For example the composition may contain at least two carriers, at least one of which is a surface-active agent.

A surface-active agent may be an emulsifying agent, a dispersing agent or a wetting agent; it may be nonionic or ionic. Examples of suitable surface-active agents include the sodium or calcium salts of polyacrylic acids and lignin sulphonic acids; the condensation of fatty acids or aliphatic amines or amides containing at least 12 carbon atoms in the molecule with ethylene oxide and/or propylene oxide; fatty acid esters of glycerol, sorbitol, sucrose or pentaerythritol; condensates of these with ethylene oxide and/or propylene oxide; condensation products of fatty alcohol or alkyl phenols, for example p-octylphenol or p-octylcresol, with ethylene oxide and/or propylene oxide; sulphates or sulphonates of these condensation products; alkali or alkaline earth metal salts, preferably sodium salts, of sulphuric or sulphonic acid esters containing at least 10 carbon atoms in the molecule, for example sodium lauryl sulphate, sodium secondary alkyl sulphates, sodium salts of sulphonated castor oil, and sodium alkylaryl sulphonates such as dodecylbenzene sulphonate; and polymers of ethylene oxide and copolymers of ethylene oxide and propylene oxide.

Pesticidal compositions may for example be formulated as wettable powders, dusts, granules, solutions, emulsifiable concentrates, emulsions, suspension concentrates and aerosols. Wettable powders usually contain 25, 50 or 75% w of active ingredient and usually contain in addition to solid inert carrier, 3-10% w of a dispersing agent and, where necessary, 0-10% w of stabiliser(s) and/or other additives such as penetrants or stickers. Dusts are usually formulated as a dust concentrate having a similar composition to that of a wettable powder but without a dispersant, and are diluted in the

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field with further solid carrier to give a composition usually containing $\frac{1}{2}$ -10% w of active ingredient.

Granules are usually prepared to have a size between 10 and 100 BS mesh (1.676-0.152 mm), and may be manufactured by agglomeration or impregnation techniques. Generally, granules will contain $\frac{1}{2}$ -75% w active ingredient and 0-10% w of additives such as stabilisers, surfactants, slow release modifiers and binding agents. The so-called "dry flowable powders" consist of relatively small granules having a relatively high concentration of active ingredient. Of particular interest in current practice are the water-dispersible granular formulations. These are in the form of dry, hard granules that are essentially dust-free, and are resistant to attrition on handling, thus minimizing the formation of dust. On contact with water, the granules readily disintegrate to form stable suspensions of the particles of active material. Such formulations contain 90% or more by weight of finely divided active material, 3-7% by weight of a blend of surfactants, which act as wetting, dispersing, suspending and binding agents, and 1-3% by weight of a finely divided carrier, which acts as a resuspending agent.

Emulsifiable concentrates usually contain, in addition to a solvent and, when necessary, co-solvent, 10-50% w/v active ingredient, 2-20% w/v emulsifiers and 0-20% w/v of other additives such as stabilisers, penetrants and corrosion inhibitors. Suspension concentrates are usually compounded so as to obtain a stable, non-sedimenting flowable product and usually contain 10-75% w active ingredient, 0.5-15% w of dispersing agents, 0.1-10% w of suspending agents such as protective colloids and thixotropic agents, 0-10% w of other additives such as defoamers, corrosion inhibitors, stabilisers, penetrants and stickers, and water or an organic liquid in which the active ingredient is substantially insoluble; certain organic solids or inorganic salts may be present dissolved in the formulation to assist in preventing sedimentation or as anti-freeze agents for water.

Aqueous dispersions and emulsions are compositions which may be obtained by diluting a wettable powder or a concentrate with water. The said emulsions may be of the water-in-oil or of the oil-in-water type, and may have a thick 'mayonnaise'-like consistency.

Pesticidal compositions may also contain other ingredients, for example further active compounds possessing herbicidal, insecticidal or fungicidal properties, in accordance with the requirements of the locus to be treated and the treatment method.

The method of applying a compound of formula I to combat termites comprises applying the compound, conveniently in a composition comprising the compound of formula I and a carrier as described above, to a locus or area to be protected from the termites, such as soil or (directly to) timber subject to or subjected to infestation or attack by termites. The compound, of course, is applied in an amount sufficient to effect the desired action. This dosage is dependent upon many factors, including the carrier employed, the method and conditions of the application, whether the formulation is present at the locus in the form of a film, or as discrete particles, the thickness of film or size of particles, and the like. Proper consideration and resolution of these factors to provide the necessary dosage of the active compound at the locus to be protected are within the skill of those versed in the art. In general, however, the effective dosage of the compound of the invention at the locus to be protected—i.e. the dosage which the

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termite contacts—is of the order of 0.001 to 0.5% based on the total weight of the composition, though under some circumstances the effective concentration may be as little as 0.0001% or as much as 2%, on the same basis.

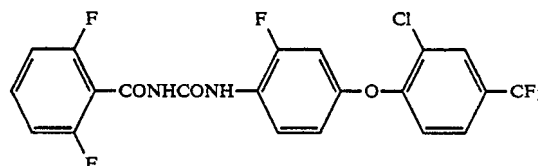
In one embodiment of this invention, acyl urea compounds of formula I are used to combat termites in the soil, thereby achieving indirect protection for any timber-based constructions erected on the treated soil. Suitable soil-based control is obtained by providing in the soil a termiticidally effective dosage of an acyl urea of formula I. For use in this manner, the acyl urea is suitably applied to the soil at a rate of from about 0.01 to about 10 kilograms per hectare. Good control of soil inhabiting termites is obtained at rates of from about 0.1 to about 5 kilograms per hectare and especially from about 0.5 to about 4 kilograms per hectare. The compound of formula I can conveniently be formulated for use as a granule or powder containing a solid diluent, impregnated with the compound, or as a suspension concentrate. Such formulations usually contain from about 1 to about 50% by weight of the compound. More effective control results when the formulation is physically lightly mixed with the topsoil. The compound of formula I can also be applied as a drench—that is, as a solution or dispersion of the compound in a suitable solvent or liquid diluent. Such drenches can be prepared by diluting with water a concentrate containing the compound of formula I, an emulsifying agent, and preferably an organic solvent, such as isophorone and/or N-methylpyrrolidone. The compound of formula I can be applied by band, furrow or side-dress techniques, and may be incorporated or not.

In another embodiment of the invention, acyl urea compounds of formula I are applied directly to timber, either before, during or after its incorporation into a building, thereby protecting it against damage from termite attack. For treatment of timber, the composition suitably contains a penetrant designed to facilitate penetration of the active ingredient to a significant depth in the timber, thereby ensuing that superficial surface abrasion will not generate a surface free from active ingredient and thus vulnerable to termite penetration. Examples of materials known for use as wood penetrants include paraffinic hydrocarbons, e.g. "Shellsol K" (trade mark) and low aromatic white spirit (LAWS), 2-ethoxyethanol, and methyl isobutyl ketone. Preferably the penetrant is 2-ethoxyethanol or methyl isobutyl ketone, optionally in association with isophorone and/or N-methyl pyrrolidone. It is useful in such timber treatment to incorporate "anti-bloom" agents, which counteract the tendency for the active ingredient to migrate to the surface ("bloom"), suitable materials being dibutyl phthalate and o-dichlorobenzene. Timber treatment compositions may also, if desired, contain fungicides (to prevent fungal attacks such as dry rot and wet rot), and/or pigments in order to combine termite protection with painting of the timber. In this context, painting will be understood to include not only the application of covering pigmentation (commonly white), but also the application of natural wood colouration in order to restore the appearance of weathered timber (e.g. as with treatments to red cedar external housing timbers). The actual application onto the timber may be carried out using conventional techniques including immersion of the timber in the liquid, and painting the liquid onto the timber by spray or brushing. The concentration of acyl urea active material in the treated timber should, of course, be sufficient to achieve

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the desired termiticidal effect. However, the total volume of formulated product taken up by the timber is limited by the absorption properties of the wood with respect to that formulation and will also vary according to the application procedure adopted (immersion or painting); hence the concentration of active ingredient in the formulation should be such as to produce the desired concentration in the treated timber. The formulation may be aqueous, as for example obtained by dilution of a conventional insecticide emulsifiable concentrate, or non-aqueous such as an undiluted emulsifiable concentrate. The organic solvent in such formulations will suitably be one of those previously described. The determination of the necessary parameters applicable to specific types of wood and particular treatment procedures can readily be determined by established techniques conventionally used by those skilled in the art. In general, however, the effective dosage of the compound in the wood may be as low as 10 ppm, with the maximum dosage dictated by cost considerations rather than biological efficacy. Often the lower dosage levels may initially give only a limited level of control, but achieve increased level of effect with more prolonged exposure.

The invention is illustrated in the following examples, all of which show the effect on the subterranean termite (*Reticulitermes santonensis*) of the compound flufenoxuron:



Example 1

Long term toxicity of impregnated wood and/or soil to mixed caste populations Treatments

Four treatment regimes were employed.

1. One untreated pine sapwood block on top of treated soil.
2. One treated pine sapwood block on top of untreated soil.
3. One treated and one untreated pine sapwood block on top of untreated soil.
4. One untreated pine sapwood block on 1 cm deep layer of treated soil on top of untreated soil.
5. Untreated wood and soil control.

Treatments 1 to 4 were applied at 2 of test compound to give 100 and 10 ppm toxicant in dry soil or dry wood.

Application

Soil treatments-3 ml of acetone solution of test compound was applied by pipette onto 20 g of dried soil (75% general compost, 25% silver sand) in 75 ml glass bottles. These were rolled on a mixer for 1 hour and then placed uncapped in a vacuum oven at ambient temperature ($21 \pm 3^\circ \text{C}$) and 500 mm mercury vacuum ($6.7 \times 10^4 \text{Pa}$) overnight to remove the acetone. All soil samples were adjusted to 20% m/m water content with tapwater and rolled again for a further 1 hour prior to infestation.

Wood treatments-pine sapwood blocks were cut $15 \times 15 \times 7 \text{ mm}$ and dried in a vacuum oven to constant

TABLE II-continued

Treatments	Replicate	% Mortality (at days after treatment)								
		1	2	3	6	8	10	13	16	27
acetone + 5 untreated workers 10 nymphs 5 ug test compound	1	0	0	0	20	20	20	20	40	100
	Adjusted	0	0	0	0	0	0	0	0	—
	1	0	0	0	0	50	60	100	100	100
	2	0	0	0	20	50	100	100	100	100
	3	0	10	10	20	50	60	70	90	100
	mean	0	3	3	13	50	73	90	97	100
+ 5 untreated workers	Adjusted mean	0	3	0	5	44	70	88	91	—
	1	0	20	20	20	20	60	60	80	100
	2	0	0	0	0	60	100	100	100	100
	3	0	0	0	0	20	20	20	20	100
	mean	0	7	7	7	33	60	60	67	100
	Adjusted mean	0	7	7	0	18	50	50	43	—

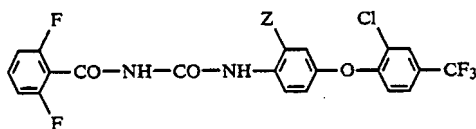
TABLE III-continued

Compound	Time after treatment	LD ₅₀ (micrograms)
(iii) comparative (Z= Cl)	3 weeks	more than 30
	2 weeks	more than 30
	3 weeks	more than 30

Example 3

Comparative topical application tests

Comparative topical application test were made using compounds of the formula



- (i) in accordance with the invention wherein Z is F (the compound being flufenoxuron),
- (ii) for comparison purposes wherein Z is H (the compound of Example 2 of UK Pat. No. 1,460,419) and
- (iii) for comparison purposes wherein Z is Cl (in accordance with UK Pat. No. 1,460,419).

Each of the three test compounds was dissolved in acetone to give solutions having concentrations of compound of 30, 10, 3, 1 and 0.3 mg/ml.

Groups of 10 workers of subterranean termite (*Reticulitermes santonensis*) were treated by topical application on the ventral abdomen of 0.5 microlitres of solution by means of a Hamilton syringe an micro-applicator. Three replicates were employed for each treatment including a blank acetone control. Post treatment, each group was held in 5 cm diameter vented plastic petri dishes containing 5 g of untreated soil (75% general compost, 25% silver sand adjusted to 10% water content) with a pine sapwood block (5×10×10 mm) as food. These petri dishes were maintained in a glass tank at 21±2° C. and 90% relative humidity throughout the test period. The soil in each dish was moistened with 0.2 ml of water once a week as this was found to improve the viability of the termites.

Assessments were carried out periodically over the following 31 days and any dead workers were removed at each evaluation. The test was discontinued at this time as sporadic fungal growth gave random mortality throughout after this period.

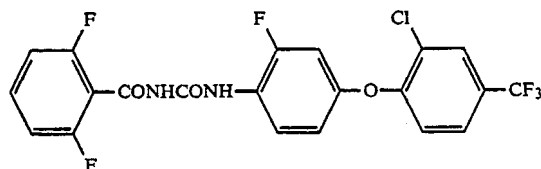
In each case an LD₅₀ (the dosage of compound required to kill half of the test species) was calculated from the mortality figures at 2 weeks and 3 weeks. Results are given in Table III following.

TABLE III

Compound	Time after treatment	LD ₅₀ (micrograms)
(i) flufenoxuron (Z = F)	2 weeks	20
	3 weeks	1
(ii) comparative (Z = H)	2 weeks	more than 30

We claim:

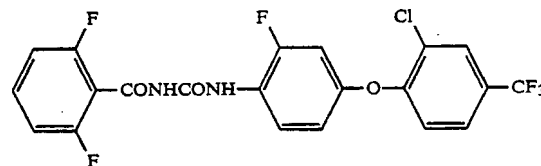
1. A method of combating termites at a locus infested with termites, said method comprising treating said locus with a termiticidally effective amount of an acyl urea of the formula



2. The method of claim 1 wherein the locus comprises soil or timber infested with termites.

3. The method of claim 2 wherein said soil or timber is treated with said acyl urea by application of a composition comprising said acyl urea and a carrier.

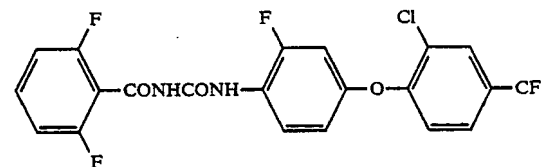
4. A method of protecting a locus subject to termite attack against such attack, said method comprising treating said locus with an amount of acyl urea compound of the formula



5. The method of claim 4 wherein said locus comprises soil or timber.

6. The method of claim 5 wherein said soil or timber is treated with said acyl urea by application of a composition comprising said acyl urea and a carrier.

7. Timber impregnated at least at a surface with a termiticidally effective amount of a termiticidally effective acyl urea compound having the formula



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