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Señor Director

División de Nuevas Creaciones

Superintendencia de Industria y Comercio

Ministerio de Comercio, Industria y Turismo

E. S. D.

Re.: Expediente No. 10 001339

Solicitud de Patente de invención para: **“DERIVADOS DE ISOTIAZOL Y PIRAZOL
COMO FUNGICIDAS”**

Solicitante: **SYNGENTA PARTICIPATIONS AG**

RESPUESTA AL EXAMEN DE FONDO

Yo, **Margarita Castellanos Abondano**, identificada como aparece al pie de mi firma, atentamente me permito presentar respuesta al oficio **No.2566** notificado por fijación en lista del 19 de febrero de 2013, estando dentro del término de la prórroga solicitada el 16 de mayo de 2013, en los siguientes términos:

De la solicitud de patente

Su Despacho concluye que el pliego reivindicatorio de la presente solicitud de patente no cumple con las disposiciones del artículo 30 de la Decisión 486. El Examinador señala lo siguiente:

- “El nuevo capítulo reivindicatorio es demasiado amplio y no cumple con el requisito de concisión; asimismo, se considera una generalización que no se encuentra enteramente soportada en la descripción. Son tantas las posibles estructuras para el compuesto de formula (I) (debido a la gran variedad de sustituyentes) que la descripción no divulga de manera

suficientemente clara y completa la materia divulgada en las reivindicaciones”.

- “Es importante indicar que no sólo no se encuentra ninguna razón técnica para tratar de proteger una extrapolación o generalización tan amplia a partir de estructuras específicas mucho más simples, sino que no se encuentra sustento en la descripción para tratar de proteger combinaciones que incluyen un ingrediente fungicida conocido adicional. Sírbase suprimir la nueva reivindicación 9 puesto que no está sustentada en la descripción”.

Con el fin de atender las observaciones formuladas a este respecto, y de acuerdo con el artículo 34 de la Decisión 486, se presenta un nuevo capítulo reivindicatorio conformado por 5 reivindicaciones como sustituto del anteriormente presentado. De tal manera que, las objeciones formuladas a este respecto se deben tener por superadas de la siguiente manera:

- Se ha limitado de forma significativa el pliego reivindicatorio a aquellos compuestos que se encuentran soportados en los ejemplos de la presente solicitud. Por lo tanto, la objeción formulada a este respecto se debe tener por superada.
- Adicionalmente, la reivindicación 9 no ha sido incluida en el nuevo pliego reivindicatorio.

Así entonces, teniendo en cuenta las modificaciones efectuadas al capítulo reivindicatorio, consideramos que las objeciones que por claridad hizo su Despacho quedan superadas ya que el nuevo capítulo reivindicatorio se encuentra redactado en los términos sugeridos por su Despacho y el mismo es ahora claro, conciso y la materia reivindicada se encuentra debidamente soportada en la descripción.

Novedad y Nivel inventivo

Su Despacho concluye que el documento WO2004/080972 (D4) afecta la novedad de las reivindicaciones 1-4 y 6-7 de la presente invención y adicionalmente, señala que los documentos WO2006031631 (D1), DE10034129 (D2) y WO0155142 (D3)

afectan el nivel inventivo de las reivindicaciones 1 a 9. En cuanto a las objeciones del examinador deseamos poner de presente lo siguiente:

De acuerdo con los artículos 16 y 18 de la Decisión 486:

“Artículo 16.- *Una invención se considerará nueva cuando no está comprendida en el estado de la técnica. El estado de la técnica comprenderá todo lo que haya sido accesible al público por una descripción escrita u oral, utilización, comercialización o cualquier otro medio antes de la fecha de presentación de la solicitud de patente o, en su caso, de la prioridad reconocida”.*

“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”.*

De conformidad con los anteriores artículos, se exponen a continuación las razones por las cuales la presente invención es novedosa frente al documento D4 e inventiva frente a las enseñanzas de los documentos D1, D2 y D3:

Novedad

Debido a que la reivindicación 1 ha sido limitada a aquellos compuestos de fórmula (I) debidamente soportados en los ejemplos de la presente solicitud y que no se encuentran anticipados por D4, es claro que la objeción formulada por novedad se debe tener por superada.

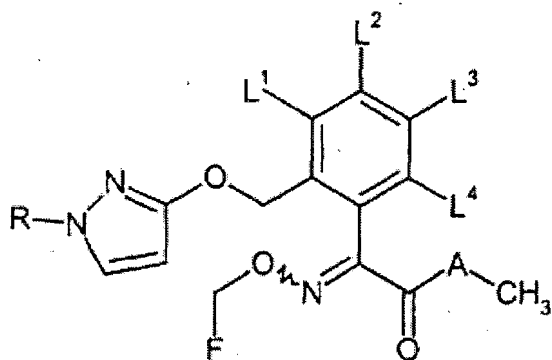
Nivel inventivo

Con respecto al documento D1 (WO 2006/031631), se presenta junto con el presente memorial datos comparativos que muestran el rendimiento mejorado de los compuestos reivindicados respecto del arte previo, en particular de D1.

En los datos comparativos presentados, se puede apreciar que los compuestos 1 y 3 de la tabla presentada corresponden a D1, mientras que los compuestos 2 y 4 corresponden a la presente invención. Queda claramente demostrado en dichos ensayos que los compuestos de la presente solicitud presentan resultados superiores a los del arte previo. Esto demuestra la existencia de altura inventiva respecto del arte previo.

De modo que, se reitera que es claro el salto tecnológico de los compuestos reivindicados frente a los conocidos en D1 y por consiguiente, se debe tener por superada la objeción formulada por nivel inventivo frente a dicho documento toda vez que la presencia de un efecto inesperado, el cual puede ser completamente diferente o representar una mejora en los resultados, es un indicio del cumplimiento del nivel inventivo.

Por otra parte, D2 (DE 10034129) se refiere a derivados de pirazolilbencileter que contienen un grupo fluormetoxiimino y su uso como pesticidas:

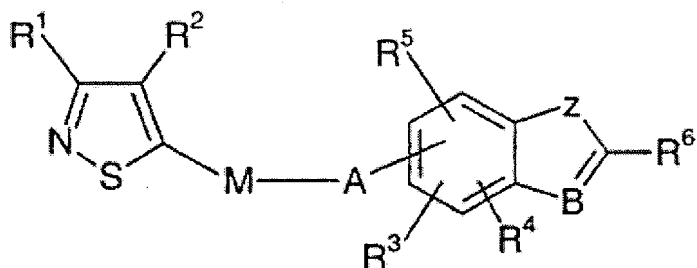


Donde R representa en cada caso alquilo, cicloalquilo o arilo opcionalmente sustituidos, el sustituyente que corresponde a R³ es un bencil eter opcionalmente sustituido, y el sustituyente correspondiente a R¹ es únicamente H.

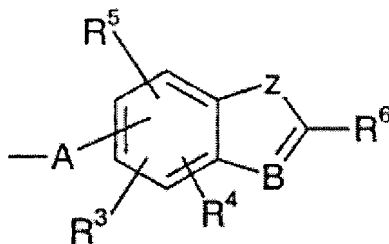
En las reivindicaciones presentes, R³ no puede ser un bencil eter opcionalmente sustituido y además R¹ no tiene el valor H. Adicionalmente, los compuestos de acuerdo con D2 presentan un H en la posición en la que la presente solicitud es un anillo pirazolilo sustituido por el grupo -CH(OR⁴)R².

De tal manera que, es claro que a partir de las enseñanzas de D2, una persona versada en la materia no habría llegado a los compuestos ventajosos de la presente solicitud de patente.

De otro lado, D3 (WO 01/155142) se refiere a derivados isotiazol y su uso como pesticidas



En el cual M significa OC (=Y), N=C (OR₈), N=C (SR₉), N=C (NR₁₀R₁₁) o N (R₁₂) C (=NR₁₃) donde O o N es el átomo de unión del grupo isotiazol y este grupo está sustituido con un grupo de acuerdo con la siguiente estructura:



En la presente solicitud, la posición correspondiente a R sólo puede ser R³ que son fenilo, tiofenilo o furanilo opcionalmente sustituidos, donde los sustituyentes opcionales son 1-2 sustituyentes seleccionados del grupo que comprende halógeno, un grupo alcoxi C₁-C₈ o alquilo C₁-C₈. Por lo tanto, en ningún caso es un grupo M sustituido con la estructura antes mencionada.

Este documento tampoco enseña que R₂ en D3 pueda ser un grupo de acuerdo con -CH(OR⁴)R² aún si eventualmente pudiera estar comprendido en el significado del radical R₂ de D3.

Asimismo, el R¹ de D3 es hidrógeno, halógeno, C₁₋₆ alquilo opcionalmente sustituido, C₂₋₆alquenilo opcionalmente sustituido, C₂₋₆alquinilo opcionalmente sustituido, C₁₋₆alcoxi opcionalmente sustituido, C₁₋₆alquiltio opcionalmente sustituido, C₃₋₇cicloalquilo opcionalmente sustituido, ciano, nitro o SF₅. En contraste, en la presente solicitud el sustituyente correspondiente debe ser aromático, es decir, arilo o heteroarilo.

De modo que, es claro que a partir de las enseñanzas del documento D3, un una persona versada en la materia no derivaría de manera obvia u evidente la presente invención.

De otro lado, vale la pena resaltar que a partir de la combinación de las enseñanzas de los documentos D1, D2 o D3 no se obtendrían los compuestos acá reivindicados toda vez que una persona versada en la materia no encontraría enseñanzas de como modificar D1 para llegar a la presente invención. En particula, a la luz del rendimiento sorprendentemente mejorado de los compuestos que se reivindican en comparación con los compuestos de D1.

Con respecto al bioisosterismo, hay numerosos estudios que informan sobre la poca relevancia de este concepto. Por ejemplo, Stringer, D. Woodcock, E. John Skerrett Insecticidal activity and chemical constitution: analogues and isosteres of ddt (<http://onlinelibrary.wiley.com/doi/10.1111/j.1744-7348.1955.tb02487.x/abstract>). En dicho documento se menciona que se sintetizaron un número de isósteros del DDT y compuestos relacionados se han sintetizado y examinado con respecto a la actividad insecticida usando grain-weevil, locustycottonstainer, entre otros. Se encuentra una marcada disminución de la actividad luego del remplazo de los átomos de cloro del DDT por grupos metilo e hidroxilo. Las conclusiones pueden resumirse de la siguiente manera: "En todos los compuestos examinados la naturaleza de los átomos o grupos unidos al carbono beta es crítica, aunque la toxicidad no parece estar relacionada cercanamente al porcentaje de similitud del rearreglo de carbono. Así, el DDT y el S. 39, que difieren considerablemente en la porción alifática de la molécula, son del mismo orden de actividad mientras que los isósteros, que son espacialmente similares, son generalmente inactivos".

Así entonces, teniendo en cuenta que las enseñanzas de las anterioridades D1, D2, D3 citadas por su Despacho por si solas o combinadas no llevan a un técnico versado en la materia a obtener los compuestos acá reivindicados toda vez que no enseñan ni sugieren como un todo las características técnicas esenciales de los mismos y que los compuestos reivindicados presentan un efecto mejorado e inesperado en comparación con compuestos estructuralmente similares del estado de la técnica, es claro que a partir de dichos documentos no era posible para un técnico medio obtener de manera obvia y/o evidente la presente invención.

Dado que la presente solicitud cumple con las disposiciones de los artículos 30, 16 y 18 de la Decisión 486, respetuosamente solicito a ese Despacho que se proceda con la concesión de la presente solicitud.

Anexos

Nuevo capítulo reivindicatorio conformado por 5 reivindicaciones.
Pruebas comparativas.

Señor Director,


Margarita Castellanos Abondano

C.C. No. 39.779.654 de Usaquén

T.P.A. No. 70.819 de C.S.J.

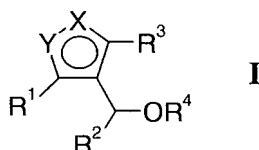
Calle 94 A No. 7 A-09 – Bogotá, D.C.

Tel.: 7560770

Bogotá D.C. - 4 JUL, 2013

REIVINDICACIONES

1. Un compuesto de Fórmula I:



donde

X es S o NR^5 e Y es N;

R^1 y R^3 son independientemente fenilo, tiofenilo o furanilo opcionalmente sustituidos, donde los sustituyentes opcionales son 1 a 2 sustituyentes seleccionados del grupo que comprende halógeno, un grupo alcoxi C_1-C_8 o alquilo C_1-C_8 ;

R^2 es piridilo o pirimidinilo;

R^4 es hidrógeno;

R^5 es alquilo C_1-C_8 o bencilo

o donde

X es N e Y es NR^5 ;

R^1 y R^3 son independientemente fenilo, tiofenilo o furanilo opcionalmente sustituidos, donde los sustituyentes opcionales son 1 a 2 sustituyentes seleccionados del grupo que comprende halógeno;

R^2 es piridilo;

R^4 es hidrógeno; y

R^5 es alquilo C_1-C_8 .

2. El compuesto de la reivindicación 1, donde

X es S e Y es N;

R^1 y R^3 son independientemente fenilo, tiofenilo o furanilo opcionalmente sustituidos, donde los sustituyentes opcionales son 1 a 2 sustituyentes seleccionados del grupo que comprende halógeno;

R^2 es piridilo o pirimidinilo y

R^4 es hidrógeno.

3. El compuesto de la reivindicación 1, donde

X es NR^5 e Y es N;

R^1 y R^3 son independientemente fenilo, tiofenilo o furanilo opcionalmente sustituidos, donde los sustituyentes opcionales son 1 a 2 sustituyentes seleccionados del grupo que comprende halógeno, un grupo alcoxi C_1-C_8 o alquilo C_1-C_8 ;

R^2 es piridilo;

R^4 es hidrógeno; y

R^5 es alquilo C_1-C_8 .

4. El compuesto de la reivindicación 1, donde

X es N e Y es NR^5 ;

R^1 y R^3 son independientemente fenilo, tiofenilo o furanilo opcionalmente sustituidos, donde los sustituyentes opcionales son 1 a 2 sustituyentes seleccionados del grupo que comprende halógeno;

R^2 es piridilo;

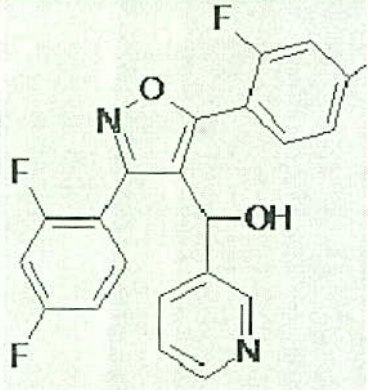
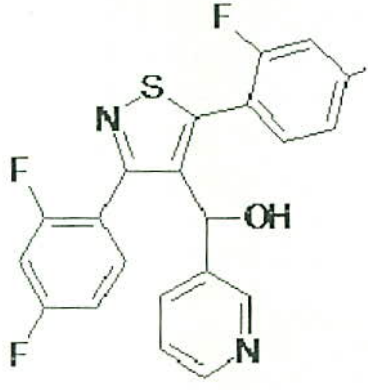
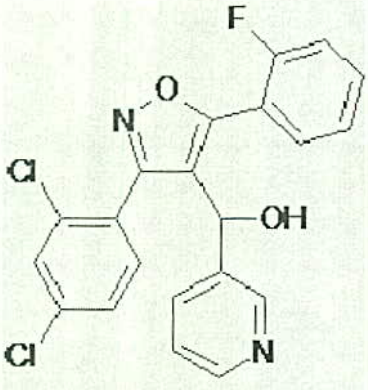
R^4 es hidrógeno y

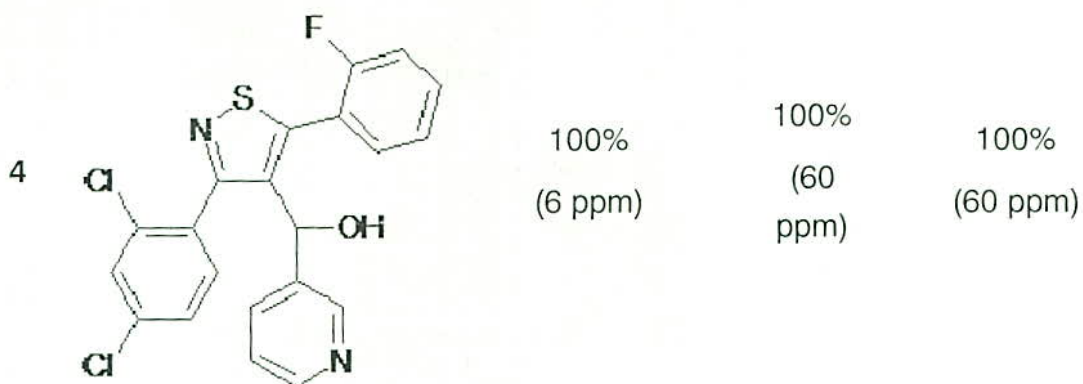
R^5 es alquilo C_1-C_8 .

5. Un método para la prevención y/o control de infecciones fúngicas en plantas y/o en material para la propagación de plantas que comprende la aplicación a la planta o material de propagación de plantas o a una parte de los mismos de una cantidad fungicida efectiva de un compuesto de fórmula I tal como se define en cualquiera de las reivindicaciones 1 a 4:

DATOS COMPARATIVOS (Ya presentados en la respuesta anterior)

Datos comparativos de los compuestos de la presente invención vs WO 2006/031631

Com - pues to		<i>Monographellan ivalis</i>	Fusariu m culmor um	<i>Rhizoctonias olani</i>
1		50% (20 ppm)	20% (20 ppm)	0% (6ppm)
2		100% (20 ppm)	100% (20 ppm)	50% (6 ppm)
3		20% (6 ppm)	50% (60 ppm)	inactivo



Procedimiento:

- ***Fusarium culmorum* (podredumbre de la raíz):** Conidios del hongo del almacenamiento criogénico se mezclaron directamente en caldo nutriente (caldo de dextrosa de papa PDB). Después de colocar una solución (DMSO) de los compuestos de ensayo en una placa de microtítulo (formato de 96 pocillos) se agregó el caldo nutriente que contenía las esporas fúngicas. Las placas de ensayo se incubaron a 24°C y se determinó la inhibición del crecimiento fotométricamente luego de 48 horas.
- ***Rhizoctoniasolani* (gomosis (footrot), mal de los almácigos (damping-off)):** Fragmentos miceliales del hongo, preparados a partir de un cultivo líquido fresco, se mezclaron directamente en caldo nutriente (caldo de dextrosa de papa (PDB, por sus siglas en inglés)). Luego de colocar una solución (DMSO) de los compuestos de ensayo en una placa de microtítulo (formato de 96 pocillos) se agregó el caldo nutriente que contenía el micelio fúngico. Las placas de ensayo se incubaron a 24°C y se determinó la inhibición del crecimiento fotométricamente luego de 48 horas
- ***Monographellanivalis* (sin. *Microdochiumnivale*, *Fusarium nivale*), moho de la nieve, gomosis de los cereales:** Los conidios del hongo del almacenamiento criogénico se mezclaron directamente en caldo nutriente (caldo de dextrosa de papa (PDB, por sus siglas en inglés)). Luego de colocar una solución (DMSO) de los compuestos de ensayo en una placa de microtítulo (formato de 96 pocillos) se agregó el caldo nutriente que contenía las esporas fúngicas. Las placas de ensayo se incubaron a 24 C y se determinó la inhibición del crecimiento fotométricamente después de 72 horas a 620nm.

STRINGER, A., WOODCOCK, D. & SKERRETT, E. J. (1955). *Ann. appl. Biol.* 43 (3), 366-378.

INSECTICIDAL ACTIVITY AND CHEMICAL CONSTITUTION: ANALOGUES AND ISOSTERES OF DDT

By A. STRINGER, D. WOODCOCK AND E. JOHN SKERRETT

Long Ashton Research Station, University of Bristol

(With 5 Text-figures)

A number of isosteres of DDT and related compounds have been synthesized and examined for insecticidal activity using the grain-weevil, locust and cotton stainer. A marked loss of activity follows replacement of the chlorine atoms of DDT by methyl and hydroxyl groups. In the nitroalkane series, the optimum structure for maximum toxicity was present in 1:1-di-*p*-chlorophenyl-2-nitropropane. The significance of these results has been reviewed in the light of current theories of DDT structure and activity. Many of the compounds expected to be toxic were found not to be so, and this inactivity can be attributed either to the initial hypotheses being invalid or to the operation of biological factors preventing concentration of the compound at the site of action.

INTRODUCTION

Busvine (1946) tested a large number of DDT analogues and concluded that the most toxic were those most similar to DDT in shape and weight. If only steric requirements are necessary for optimal toxicity then replacement of chlorine atoms by spatially similar atoms should not affect the activity and the isosteres should act similarly to DDT. A number of compounds isosteric with DDT were accordingly synthesized in which the dimensions of the hydroxyl group (radius 1.7 Å) or methyl group (radius 2.0 Å) agree reasonably well with those of the chlorine atom (radius 1.8 Å) which they replaced (Skerrett & Woodcock, 1950, 1952*a, b*). This degree of structural conformity is well within the limits required for isosteric activity in serological reactions (Pauling & Pressman, 1945).

Since there was not such a complete electrical analogy between these isosteres and DDT, it was suggested that the high insecticidal activity of DDT might in some way be associated with the presence of strong electrophilic groups in the aliphatic portion of the molecule (Skerrett, Stringer & Woodcock, 1951), and therefore a number of di-*p*-chlorophenyl-alkanes fulfilling these conditions, were prepared (Skerrett & Woodcock, 1952*c*).

MATERIALS

The compounds used in this investigation are listed below, the original code numbers being given so as to facilitate subsequent reference to them.

TABLE I

Code no.	Reference	Compound
Isosteres		
S. 5	<i>a</i>	1:1-Di- <i>p</i> -chlorophenyl-2:2-dimethylpropane
S. 26	<i>a</i>	1:1-Di- <i>p</i> -chlorophenyl-2-methylpropan-2-ol
S. 27	<i>a</i>	2-Chloro-1:1-di- <i>p</i> -chlorophenyl-2-methylpropane
S. 59	<i>a</i>	1- <i>p</i> -Chlorophenyl-1- <i>p</i> -tolyl-2:2-dimethylpropane
S. 6	<i>a</i>	1:1-Di- <i>p</i> -tolyl-2:2-dimethylpropane
S. 64	<i>a</i>	1:1-Di- <i>p</i> -tolyl-2-methylpropan-2-ol
S. 68	<i>a</i>	2-Chloro-1:1-di- <i>p</i> -tolyl-2-methylpropane
S. 85	<i>b</i>	1:1-Di- <i>p</i> -methoxyphenyl-2:2-dimethylpropane
S. 99	<i>f</i>	1:1-Di- <i>p</i> -methoxyphenyl-2-methylpropan-2-ol
S. 146	—	1:1-Di- <i>p</i> -tolyl-2:2:2-trichloroethane
S. 147	—	1- <i>p</i> -Chlorophenyl-1- <i>p</i> -tolyl-2:2:2-trichloroethane
S. 148	—	1:1-Di- <i>p</i> -methoxyphenyl-2:2:2-trichloroethane
Nitro compounds		
S. 39	<i>d</i>	1:1-Di- <i>p</i> -chlorophenyl-2-nitropropane
S. 52	<i>c</i>	1:1-Di- <i>p</i> -chlorophenyl-2-nitroethane
S. 113	<i>c</i>	1-Chloro-2:2-di- <i>p</i> -chlorophenyl-1-nitroethane
S. 136	<i>c</i>	1:1-Dichloro-2:2-di- <i>p</i> -chlorophenyl-1-nitroethane
S. 88	<i>c</i>	2-Chloro-1:1-di- <i>p</i> -chlorophenyl-2-nitropropane
S. 82	<i>c</i>	1:1-Di- <i>p</i> -chlorophenyl-2-propylamine
S. 138	<i>d</i>	1:1-Di- <i>p</i> -chlorophenyl-2-nitrobutane
Additional compounds		
—	—	1:1-Diphenyl-2:2:2-trichloroethane
S. 55	<i>e</i>	2:2-Dimethyl-1:1-di- <i>p</i> -tolylpropan-1-ol
S. 65	<i>e</i>	1- <i>p</i> -Chlorophenyl-1- <i>p</i> -tolyl-2:2-dimethylpropan-1-ol
S. 35	<i>e</i>	Bis- <i>p</i> -chlorophenylacetamide
S. 95	—	Cyano-bis- <i>p</i> -methoxyphenylmethane
S. 36	<i>a</i>	Cyano-bis- <i>p</i> -chlorophenyl methane
S. 133	<i>a</i>	2:2-Dimethyl-1:1-diphenylpropane
S. 100	<i>c</i>	1:1-Di- <i>p</i> -chlorophenylethan-2-ol
S. 141	<i>e</i>	1:1-Di- <i>p</i> -chlorophenyl-2-methylprop-2-ene
S. 51	<i>e</i>	1:2-Dichloro-1:1-di- <i>p</i> -chlorophenyl-2-methylpropane
S. 46	<i>e</i>	1-Chloro-1:1-di- <i>p</i> -chlorophenyl-2:2-dimethylpropane
S. 98	<i>c</i>	<i>N</i> -(1:1-Di- <i>p</i> -chlorophenyl-2-propyl)-trimethyl-ammonium iodide

a Skerrett & Woodcock (1952*a*).

b Supplied by Drs Brown and Rogers, Merck and Co. Inc., Rahway, New Jersey, U.S.A.

c Skerrett & Woodcock (1952*c*).

d Supplied by the Insecticide and Fungicide Co-ordination Service of the Agricultural Research Council.

e Skerrett & Woodcock (1950).

Skerrett & Woodcock (unpublished).

TECHNIQUE

The test insects used were adult grain weevils (*Calandra granaria* L.), adult male cotton stainers (*Dysdercus fasciatus* Sign.), and adult male locusts (*Locusta migratoria migratorioides* Reiche & Fairm.).

Batches of fifty *Calandra* adults were enclosed, by means of glass rings, on filter-papers which had been treated with insecticide (Stringer, 1949*b*). Initial screening

tests were carried out using crystalline deposits from acetone, at 5 mg. per filter-paper (7 cm. diam.). Many of the compounds tested under these conditions were inactive, but those showing toxicity were then assayed using oil films; in both cases a 24 hr. exposure period, followed by a 6-day recovery period, was used. Thus the percentage mortalities can be considered as end-point observations (Beard, 1949).

Dysdercus adults, age 4-5 days, were treated individually, and then kept for 6 days in small glass containers; soaked cotton seeds were also included so that the insects could feed. It was found that the doses required were too small to make the use of acetone solutions practicable. DDT and similar compounds are highly soluble in vegetable oils and so arachis oil was used. In preliminary experiments it was noticed that when oil solutions were placed on the ventral surface of the abdomen or between the coxae the insect bent its proboscis up to the treated areas and drank part of the oil. Optimal experimental doses were found to be of the

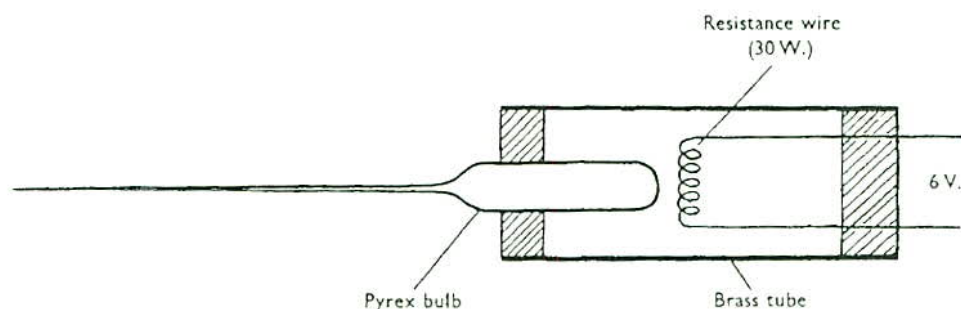


Fig. 1. Diagrammatic section of micropipette.

order of 0.1-0.2 μ l. placed on the head and neck. To facilitate a reasonably rapid method of placing these small quantities a micropipette was constructed in which the amount being delivered was directly visible. The pipette (Fig. 1) consisted essentially of a Pyrex glass bulb, made from 7 mm. tubing, drawn out into a long capillary, external diameter 230-240 μ , with the tip tapered to a blunt point. A mark was made on the glass needle approximately 1 cm. from the tip. The needle is filled to the mark by capillary action and then ejected by the expansion of the air when the bulb is heated by the coil. Calibration is carried out by ejecting the measured doses of oil on to a cotton thread suspended from a torsion balance. Readings obtained had an error of less than 10 μ g. on doses varying from 120 to 160 μ g.

Locusta adults were treated topically with 10 μ l. doses of acetone solution or by injections of 5 μ l. aqueous suspension into the abdominal body cavity. The micropipette used was a modified version of the microlitre burette described by Linderström-Lang & Holter (1933), and is similar to the one used by Heal & Menusan (1948). The standard error of 10 μ l. doses was 2%.

RESULTS

DDT and the compounds listed in Table 1 were surprisingly non-toxic when tested against *Locusta*. DDT at 100 μ g., either from acetone solution or in P-31 oil, had no apparent action when applied externally on the abdomen, thorax, head or legs. The same dose injected into the body cavity of the abdomen, as oil solution or aqueous suspension, was also non-toxic. The compounds of Table 1 were also non-toxic at 100 μ g. on the abdominal sternites. DDT was still inactive when the applied dose was increased to 1000 μ g. on the abdomen.

The relative potencies of DDT, isosteres, and nitro-compounds tested against *Calandra* are given in Table 2. The compounds listed in Table 1 and not given in Table 2 were all assayed but found to possess negligible or no toxicity. The regression slopes in Table 2 are respectively 1.81 ± 0.14 , 1.82 ± 0.11 , 1.72 ± 0.12 , and 2.07 ± 0.15 ; the difference between them is not significant.

The dosage-mortality data for the isosteres and nitro-alkanes against *Dysdercus* are given in Table 3. The slopes of the two groups, 3.83 ± 0.24 and 4.28 ± 0.26 , are not significantly different. The toxicities of five of the compounds examined were so low that their relative potencies against DDT could only be determined by extrapolation of their probit curve, on the assumption that they were parallel to the DDT regression line. Thus S. 27, S. 68, S. 59, S. 64 and S. 136 were assigned potency values of 3, 6, 3, 5 and 5 relative to 100 for DDT. Isosteres S. 6, S. 26, S. 99, nitro-alkanes S. 52, S. 82, and compounds S. 100, S. 133, S. 141 had negligible or no activity.

In Figs. 2-5, the area of the bar above the rectangle enclosing each structural formula represents the relative potency, which is also expressed numerically at the lower left-hand corner of the rectangle. The right-hand figure is the code number.

DISCUSSION

The test insects showed marked differences of susceptibility, *Locusta* being completely insensitive, *Calandra* moderately resistant, and *Dysdercus* relatively susceptible. Experiments on the lack of contact activity against the locust seem to suggest that DDT does not pass through the cuticle and that the epidermis is probably the barrier preventing entry into the body (Stringer, unpublished data). The non-toxicity of DDT injected into the body cavity has not yet been investigated, but it may possibly be due to an efficient method of detoxification or to the protection of the nervous system by the large amounts of overlying fatty tissue.

The dosage tolerance distributions for *Dysdercus* and *Calandra* are not really comparable since two types of bioassay were used and the differences may not be entirely due to species difference. But in each species the tolerance variance remains reasonably constant for all the compounds which showed activity. In spite

TABLE 2. *Calandra granaria*. Dosage-mortality data

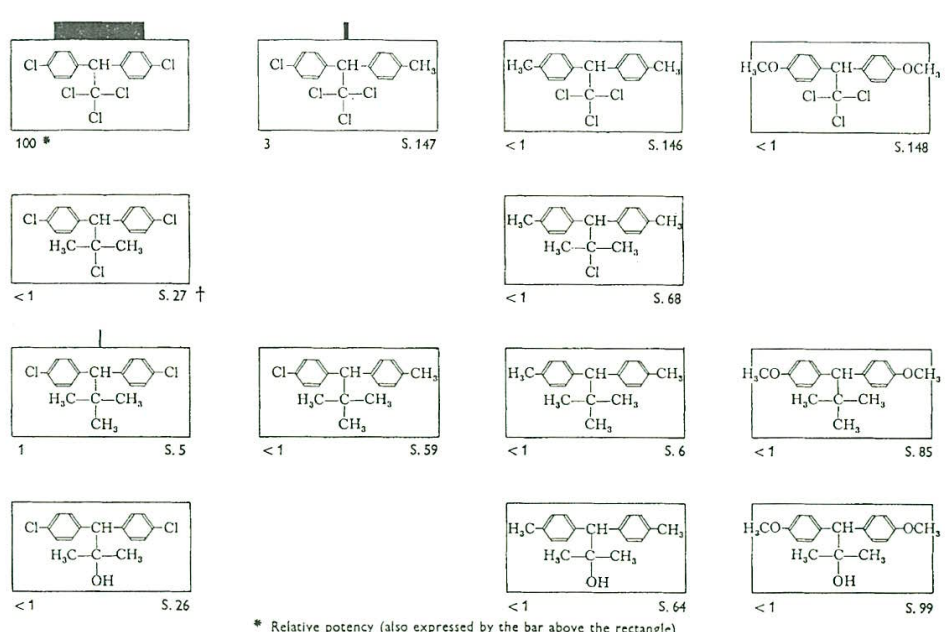
Code no.	Compound	Probit equation	<i>m</i>	<i>M</i>	Relative potency	5 % fiducial limits	Analysis of χ^2			
								D.F.	Sum of squares	Probability
DDT	1:1-Di- <i>p</i> -chlorophenyl-2:2:2-trichloroethane	$Y = 1.81x + 1.492$	1.943	—	100	—	Parallelism of regressions	1	1.297	c. 0.3
S. 147	1- <i>p</i> -Chlorophenyl-1- <i>p</i> -tolyl-2:2:2-trichloroethane	$Y = 1.81x - 1.206$	3.438	-1.496	3.2	2.6 to 3.9	Residual heterogeneity	6	5.800	—
							Total	7	7.187	—
DDT	1:1-Di- <i>p</i> -chlorophenyl-2:2:2-trichloroethane	$Y = 1.82x + 2.053$	1.620	—	100	—	Parallelism of regressions	1	1.514	c. 0.2
S. 39	1:1-Di- <i>p</i> -chlorophenyl-2-nitropropane	$Y = 1.82x + 2.516$	1.366	0.255	180	135 to 239	Residual heterogeneity	9	6.875	—
							Total	10	8.389	—
S. 39	1:1-Di- <i>p</i> -chlorophenyl-2-nitropropane	$Y = 1.72x + 1.933$	1.779	—	100	—	Parallelism of regressions	1	0.124	c. 0.7
S. 138	1:1-Di- <i>p</i> -chlorophenyl-2-nitrobutane	$Y = 1.72x + 1.305$	2.143	-0.3641	78	57 to 106	Residual heterogeneity	10	3.831	—
							Total	11	3.955	—
DDT	1:1-Di- <i>p</i> -chlorophenyl-2:2:2-trichloroethane	$Y = 2.07x - 0.405$	2.617	—	100	—	Parallelism of regressions	1	0.341	c. 0.6
S. 88	2-Chloro-1:1-di- <i>p</i> -chlorophenyl-2-nitropropane	$Y = 2.07x + 0.294$	2.279	-0.338	46	34 to 61	Residual heterogeneity	8	5.308	—
							Total	9	5.649	—

($x = \log (\text{mg./ml.}) + 2.$)

TABLE 3. *Dysdercus fasciatus*. Dosage-mortality data

Code no.	Compound	Probit equation	<i>m</i>	<i>M</i>	Relative potency	5 % fiducial limits	Analysis of χ^2			
								D.F.	Sum of squares	Probability
DDT	1:1-Di- <i>p</i> -chlorophenyl-2:2:2-trichloroethane	$Y = 3.83x + 1.101$	1.019	—	100	—				
S. 5	1:1-Di- <i>p</i> -chlorophenyl-2:2-dimethylpropane	$Y = 3.83x - 2.247$	1.894	-0.875	13	11 to 17	Parallelism of regressions	5	1.39	0.9 to 0.95
S. 85	1:1-Di- <i>p</i> -anisyl-2:2-dimethylpropane	$Y = 3.83x - 1.098$	1.593	-0.574	27	21 to 33	Residual heterogeneity	7	5.52	0.5 to 0.7
S. 146	1:1-Di- <i>p</i> -tolyl-2:2:2-trichloroethane	$Y = 3.83x - 0.689$	1.487	-0.468	34	27 to 43	Total	12	6.91	—
S. 147	1- <i>p</i> -Chlorophenyl-1- <i>p</i> -tolyl-2:2:2-trichloroethane	$Y = 3.83x + 0.089$	1.283	-0.264	55	48 to 69				
S. 148	1:1-Di- <i>p</i> -anisyl-2:2:2-trichloroethane	$Y = 3.83x + 2.136$	0.748	0.271	187	150 to 232				
DDT	1:1-Di- <i>p</i> -chlorophenyl-2:2:2-trichloroethane	$Y = 4.28x + 0.428$	1.068	—	100	—				
S. 113	2-Chloro-1:1-di- <i>p</i> -chlorophenyl-2-nitroethane	$Y = 4.28x - 1.589$	1.539	-0.471	34	28 to 41	Parallelism of regressions	4	5.29	0.2 to 0.3
S. 88	2-Chloro-1:1-di- <i>p</i> -chlorophenyl-2-nitropropane	$Y = 4.28x - 0.147$	1.203	-0.135	73	60 to 90	Residual heterogeneity	11	12.15	0.3 to 0.5
							Total	15	17.44	—
S. 39	1:1-Di- <i>p</i> -chlorophenyl-2-nitropropane	$Y = 4.28x + 0.982$	0.939	0.129	135	112 to 162				
S. 138	1:1-Di- <i>p</i> -chlorophenyl-2-nitrobutane	$Y = 4.28x + 0.425$	1.069	-0.001	100	82 to 121				

($x = \log (\text{mg.}) + 1.$)



* Relative potency (also expressed by the bar above the rectangle)
† Code number
Fig. 2. Relative potencies of DDT isosteres to *Calandra granaria*.

of the different systems used, it would appear that the susceptibility of *Calandra* to the isosteres is low when compared with that of *Dysdercus*, whereas the toxicities of the nitro-compounds are of the same order of effectiveness relative to DDT. The

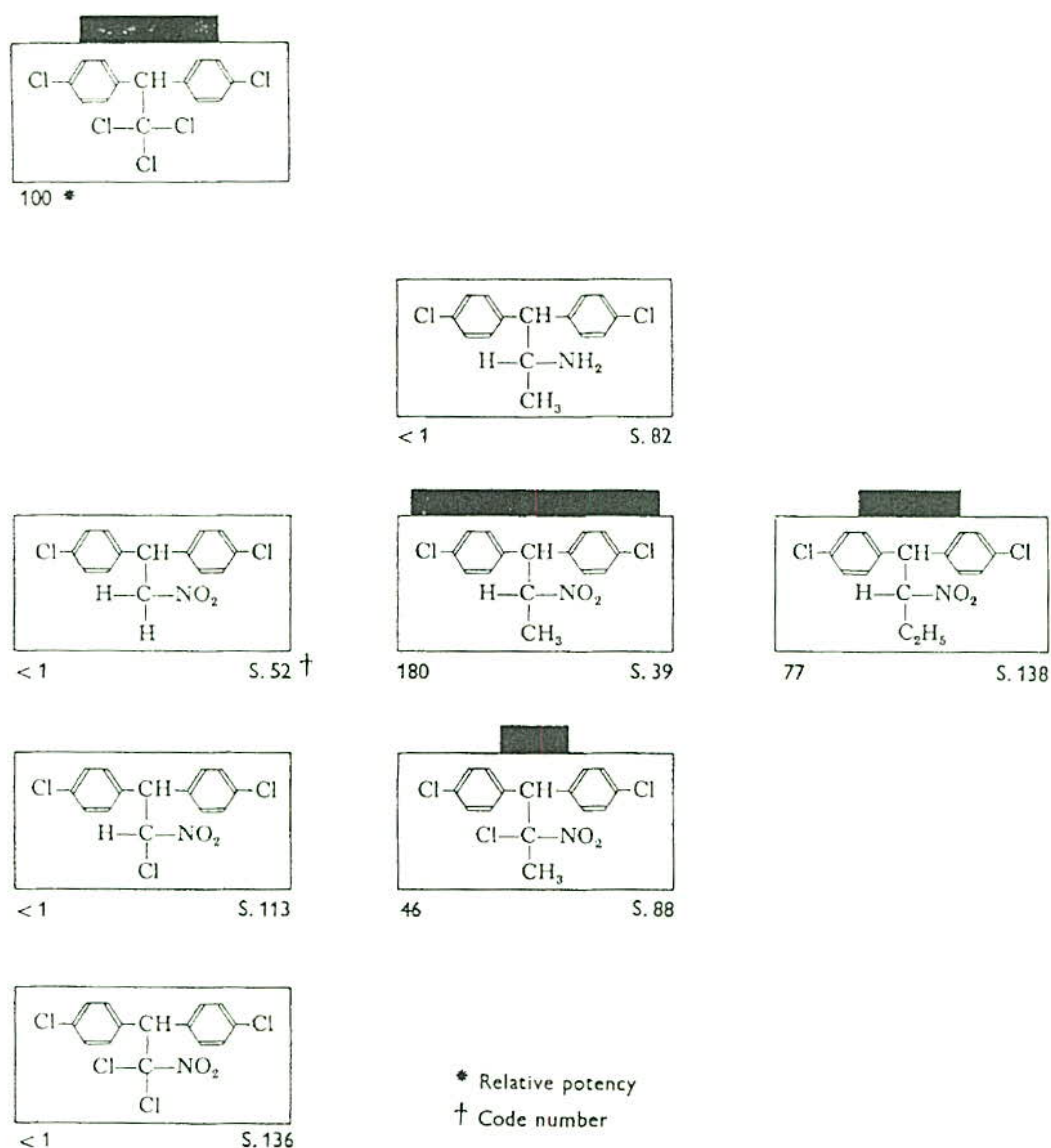


Fig. 3. Relative potencies to *Calandra granaria* of compounds related to DDT.

parallelism of the probit regression lines indicates similar tolerance variances, and in view of the fact that all the compounds are either isosteric or closely related to DDT, it is reasonable to assume that the activity, if any, is fundamentally due to the same mode of action.

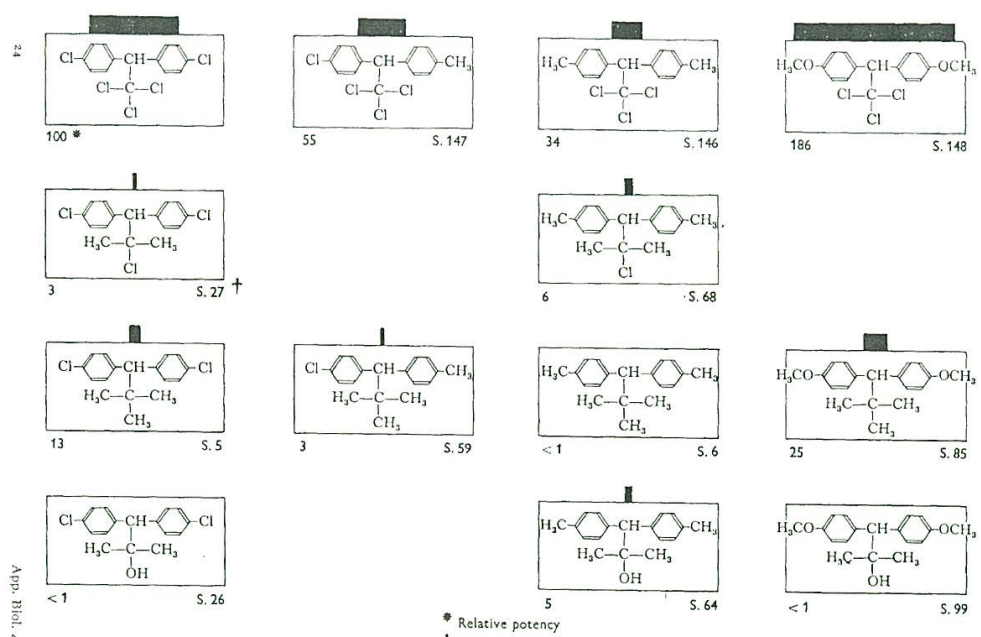


Fig. 4. Relative potencies of DDT isosteres to *Dysdercus fasciatus*.

App. Biol. 43

The results obtained in the bioassays represent the relative potencies or comparative insecticidal effectiveness and are not necessarily the relative activities at the site of action. At present, action on the biological ultrastructure is mainly

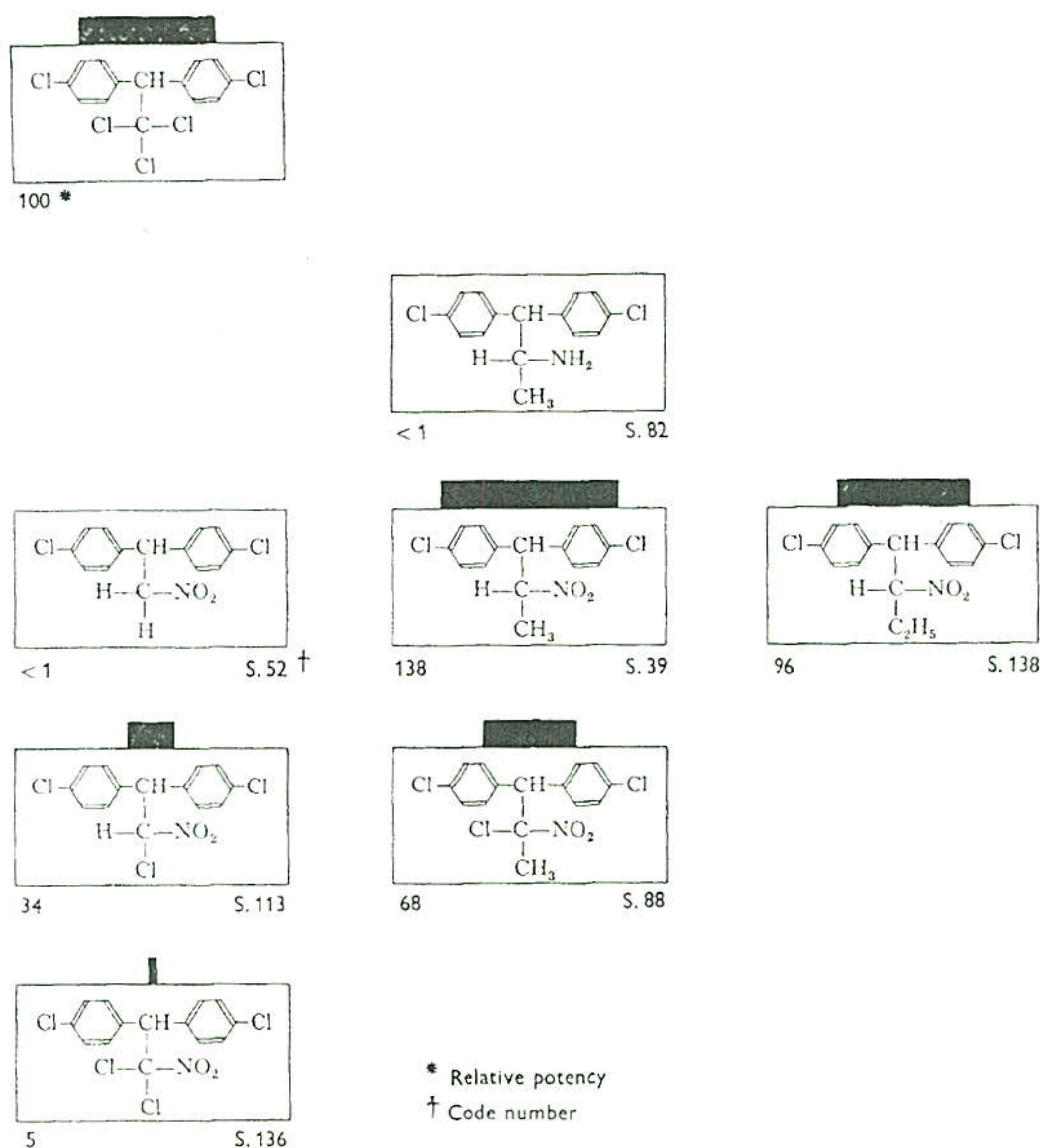


Fig. 5. Relative potencies of compounds related to DDT to *Dysdercus fasciatus*.

speculative and the associated factors concerning translocation, accumulation in various tissues, and rate of detoxification are also insufficiently known. Hypotheses concerning fundamental activity are, therefore, not necessarily disproved because

the data obtained from estimates of effectiveness, expressed as functions of the applied dosage, are not in agreement with them.

Gunther, Blinn, Carman & Metcalf (1954) have recently postulated that DDT-type molecules fit into a cavity in an apoenzyme or other protein and being held there, act by enzyme inhibition or protein interaction. They suggest that a closely fitting and tightly held molecule is a pre-requisite of a good insecticide; larger molecules which have difficulty in entering the cavity and molecules which are too small will be inactive. This emphasis on the size of the molecule has been made by several workers, but Gunther *et al.* extended the concept by claiming that since the van der Waals radii of the chlorine atom and the methyl group differ so little, their insecticidal equivalence should be comparable with the magnitudes of their van der Waals forces of attraction for the protein. If this is true then the isosteres of DDT would be expected to have activities of the same order as DDT.

It is immediately obvious from the results tabulated in Figs. 2 and 4 that the isosteres are considerably less effective than DDT. It is possible that the difference of effectiveness is due to non-accumulation at the site of action because of differences of lipid solubilities, partition coefficients, etc., but the non-toxicity of so many isosteres makes the Gunther hypothesis suspect. The nitro-compounds S. 39, S. 138 and S. 88 are of the same order of toxicity as DDT, and apparently act similarly, but they are not isosteric with DDT (Figs. 3 and 5).

The lack of activity in the isosteres of DDT led to the suggestion that the high activity of DDT may be associated with the presence of strong electropositive groups on the β -carbon atom (Skerrett *et al.* 1951). The high toxicity of nitro-compounds S. 39, S. 138 and S. 68 supports this hypothesis, but the low or negligible activity of S. 52, S. 113 and S. 136 presents difficulties. The DDT analogue containing the strong electrophilic $-\text{CF}_3$ group has been shown to possess very little activity (Kirkwood & Dacey, 1946) and it was postulated that this was probably due to alteration in the lipid affinity of the animal concerned (Kirkwood & Phillips, 1946). Again we have the confusion of being unable to separate toxicity and effectiveness, so that it is not possible to ascertain whether the poor insecticidal properties of the nitroalkanes S. 52, S. 113 and S. 136 are due to intrinsic low toxicity or other factors.

1:1-Di-*p*-chlorophenyl-2-nitroethane (S. 52) was described as 'an efficient insecticide' in the original patent and later to be 'particularly effective' against houseflies (Müller, 1946); in our investigation it proved to be completely non-toxic against all three test insects.* Successive chlorinations give the monochloro- and the dichloro-compounds (S. 113 and S. 136), which possessed little or no toxicity against *Calandra* and *Dysdercus*. This is surprising in view of the close spatial and electrical similarity between the dichloro-compound (S. 136) and DDT. If toxicity is associated with the presence of electrophilic groups on the β -carbon atom then an

* LD_{50} at 25° C. against female houseflies (Staufferstrain) in Hoskins, Witt & Erwin's vial test (1952) was 410 $\mu\text{g.}$ against that of 2 $\mu\text{g.}$ for DDT (Woodcock, unpublished data).

increased activity would be expected with the chlorination of the nitro-propane (S. 39) to the nitro-propylchloride derivative (S. 88), but a decrease in insecticidal activity was found. This result corresponds with the inactivity of the chloroethanes (S. 113 and S. 136) and throws some doubt on the validity of the suggestion concerning the β -carbon electrophilic groups. It is possible that there is an optimum electronic density about the β -carbon atom of the aliphatic part of the molecule which determines lipid solubilities and other factors concerned in translocation and distribution of the compound in the biophase.

Compounds S. 113 and S. 136 are isosteric with S. 39 and S. 88, the methyl group being replaced by a chlorine atom. The loss of activity caused by the introduction of chlorine on the β -carbon atom is unexpected in view of previous findings in the case of DDT analogues and chlorinated 4-chlorophenylethanes (Stringer, 1949a).

In a recent paper (Rogers, Brown, Rasmussen & Heal, 1953), it is suggested that the shape of the DDT molecules is of secondary importance and that the activity of DDT-type compounds can be correlated with a trihedralized configuration of the molecule. The essential feature of this conception is that the bulky trichloromethyl group exerts a steric effect causing the aromatic rings to approach coplanarity. Any other bulky substituent on the β -carbon atom similarly ought to give an effective insecticide, provided that the function of the trichloromethyl group, other than trihedralization, are also fulfilled. This theory provides an explanation for certain changes in activity which follow an alteration in size of the substituents on the β -carbon atom. Thus in the series $R_2CH.CCl_3$, $R_2CH.CHCl_2$, $R_2CH.CH_2Cl$ and $R_2CH.CH_3$ (where $R = p$ -chlorophenyl), there is a gradual decrease in activity (Stringer, 1949a). The high activity of S. 39 and S. 138 compared with the simpler S. 52 can be explained by the prevention of rotation by the introduction of the methyl and ethyl groups, and the resulting coplanarity of the p -chlorophenyl groups. On the other hand, the low activity of S. 113, compared with the high activity of the nitro-propane (S. 39) which is spatially equivalent, does not support this hypothesis. Other anomalies include the complete inactivity of compounds such as 1:1-di- p -chlorophenyl-2:2-dichloroethylene (DDX) and, more seriously, the negligible activity of the isosteres.

The views of Rogers *et al.* have also been criticized by Riemschneider (1954), largely because the trihedralization theory fails to explain the activity of different DDT analogues, or more strictly—isomers. Following a survey of twenty-five DDT analogues using molecular models Riemschneider concluded that all compounds in which a certain hindrance of free rotation of molecular groups was apparent, possessed little or no insecticidal activity. He considers the isosteres ('relatively distantly related DDT analogues') to be intrinsically inactive or at most weakly active.

CONCLUSIONS

The marked loss of activity among the DDT isosteres consequent upon the successive replacement of the chlorine atoms by methyl or hydroxyl groups, is well shown by several series in Fig. 4. Apart from the enigmatic effect of methoxyl groups as *p*-substituents, which is obvious from a consideration of Figs. 2 and 4, maximum toxicity in this group is exhibited by DDT itself.

In the nitro-alkane series, the optimum structure for maximum toxicity is present in 1:1-di-*p*-chlorophenyl-2-nitropropane (S. 39). Derived and related structures are less toxic, and loss of toxicity cannot be satisfactorily explained by current theories of structure and activity.

In all compounds examined the nature of atoms or groups attached to the β -carbon atom is critical, although the toxicity does not appear to be closely related with similarities of β -carbon arrangement. Thus DDT and S. 39, differing considerably in the aliphatic portion of the molecule, are of the same order of activity whilst the isosteres, spatially similar, are generally inactive.

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378 A. STRINGER, D. WOODCOCK AND E. J. SKERRETT

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