Rapid Paper

Insecticidal Amides with Selective Potency against a Resistant (*Super-kdr*) Strain of Houseflies (*Musca domestica* L.)

Michael ELLIOTT,[†] Andrew W. FARNHAM,
Norman F. JAMES,* Diana M. JOHNSON,
David A. PULMAN^{††} and Roman M. SAWICKI

Department of Insecticides and Fungicides,
Rothamsted Experimental Station,
Harpenden, Herts. AL5 2JQ, U.K.

[†]Pesticide Chemistry and Toxicology Laboratory,
Department of Entomological Sciences,
115 Wellman Hall, University of California,
Berkeley, California 94720, U.S.A.

^{††}Wellcome Research Laboratories, Ravens Lane,
Berkhamsted, Herts. HP4 2DY, U.K.

Received November 28, 1985

Insecticidal synthetic amides related to a novel lead compound, (2*E*,4*E*)-*N*-isobutyl-6-phenyl-hexa-2,4-dien-1-amine, and to some natural isobutylamides are up to four times as effective for both knockdown and kill against houseflies with the *super-kdr* mechanism of resistance (which operates against DDT and pyrethroids) than against the *S*-strain. This selectivity in the response to *N*-alkylamides demonstrates that the non-metabolic resistance mechanism, *super-kdr*, is vulnerable to and might be combated effectively with *N*-alkylamides.

Although the insecticidal activity of amides from *Piper* species has been recognized for some 50 years,¹⁾ the relationship between their structures and activities has remained obscure since many isolated compounds were unstable and poorly characterised. Many of the active compounds have an *N*-isobutyl group, but no other common structural feature is easily discernible. Recent investigations^{2~4)} have shown the possibility of well-characterised activity in limited areas. Following preliminary investigations of a range of synthetic analogues with various, sometimes tenuous, connections with known compounds, the active compound (IIa)

was identified as a suitable lead structure for a wide-ranging study. The synthesis of this compound was prompted by the possible relationship with the weakly active compound, tetrahydroanacyclin (I, see Fig. 1),⁶⁾ in which the acyclic diene system was envisaged as equivalent to a benzene ring, an approach successful in the pyrethroid field.⁷⁾ Our studies⁸⁾ have established many new structure-activity relationships and led to a series of active compounds, of which II (a~g) (Fig. 1) are representative. They are as effective as many other recognised insecticides in topical application tests.

MATERIALS AND METHODS

Compounds. Compounds II (a~g) were formed through Wadsworth-Emmons type condensations between an aryl acetaldehyde and a diethyl 3-(alkylcarbamoyl) allylphosphonate as described.⁸⁾

Bioassay. Topical application of measured drops (1 μ l) in acetone to adult houseflies (*Musca domestica* L.) was

FIG. 1. Structures of Compounds Discussed.

* To whom all correspondence should be addressed.

TABLE I. RESULTS FOR SUSCEPTIBLE AND RESISTANT HOUSEFLIES COMPARED

	S strain	<i>Super-kdr</i> strain	"Resistance factor" (<i>super kdr</i> /S)
LD ₅₀ s:			
Bioresmethrin	0.0039 ^a	0.15	38
Deltamethrin	0.00044	0.17	390
DDT	0.046	1.5	33
Ila	0.33	0.21	0.64
Ilb	0.098	0.063	0.64
Ilc	0.66	0.16	0.24
Ild	0.37	0.16	0.43
Ile	0.13	0.043	0.33
IIf	0.12	0.040	0.33
Ilg	0.22	0.11	0.50
Synergised LD ₅₀ s:			
IIf + PB	0.022	0.0057	0.26
IIf + PPPP	0.018	0.0063	0.35
Ile + PB	0.033	0.0067	0.20
Ile + PPPP	0.026	0.0058	0.22
Deltamethrin + PPPP	0.000058	0.020	340
Joint action with pyrethroids. KT50's:			
Bioresmethrin (0.01%)	15 min	(0.025%)	> 30 min
Ile (0.004%)	> 30 min	(0.004%)	> 30 min
Together	15 min		> 30 min
Ile (0.04%)	11.5 min	(0.04%)	7 min
Bioresmethrin (0.005%)	> 30 min	(0.15%)	> 30 min
Together	11.5 min		6.6 min

^a LD₅₀s are given in µg per fly.

followed by assessment of mortality after 48 hr, and probit analysis of the results to obtain LD₅₀s. The full details are given elsewhere.⁹⁾

The S and *super-kdr* strains of houseflies have been fully characterised and described.¹⁰⁾

Synergists. Piperonyl butoxide (PB) and *n*-propyl prop-2-ynyl phenylphosphonate (PPPP) were applied at 1 µg and 0.5 µg per fly, respectively, simultaneously with the insecticide.

Knockdown tests. The method, based on an early description,¹¹⁾ involved a modified Kearns & March spray chamber. The time for 50% of the population to be knocked down (KT50) was computed after assessment of immobilised flies at fixed intervals.

RESULTS AND DISCUSSION

The results in Table I show the compounds to be consistently up to four times more effective against houseflies with the *super-kdr* mechanism of resistance than against a sus-

ceptible (S) strain. This unusual negative cross-resistance suggests that *N*-alkylamides could combat resistance caused by *super-kdr* which in adult houseflies delays the onset of knock-down and decreases kill by DDT and its analogues, and by pyrethroids.¹⁰⁾ *Kdr* resistance, which is non-metabolic, and therefore unaffected by the usual DDT or pyrethroid synergists,¹²⁾ is associated with decreased sensitivity of the insect nervous system to these insecticides at the level of the Na⁺ channel within the lipophilic environment of the membrane adjacent to or near the Na⁺ gate protein.¹³⁾ The differential toxicity of *N*-alkylamides between the S and *super-kdr* strains was maintained after the flies had been treated with synergist. In consequence, the synergised LD₅₀ for Ile against the *super-kdr* strain is about a quarter that of the most effective pyrethroid/synergist combination.

N-Isobutylundecylenamide¹⁴⁾ synergises pyrethrins, so possible synergism between *N*-alkylamides and pyrethroids was examined. When a sublethal dose of one of the two compounds (bioresmethrin or IIe) was added to an effective dose of the other, there was no observable mutual synergism against either strain for knockdown (Table I). These spray tests also demonstrated that IIe is not only an effective killing agent, but also produces knockdown, particularly against houseflies with *super-kdr*. Thus the presence of this major pyrethroid/DDT resistance mechanism was associated with increased sensitivity to the *N*-alkylamide throughout the course of the toxic response.

Recent neurophysiological work¹⁵⁾ using a housefly nerve preparation indicated that the weakly insecticidal *N*-alkylamide (III), (2*E*,6*Z*,8*E*)-*N*-isobutyl-9-phenylnona-2,6,8-trienamide (LD₅₀ 40.4 µg per fly), causes spontaneous repetitive activity followed by depression leading to nerve block, with little evidence of synaptic action. This resembles the effects of "type I" pyrethroids and DDT.¹⁶⁾ However, (III) by itself has no effect on synaptosomes,¹⁵⁾ but strongly antagonizes the stimulatory effect of veratridine which induces the release of choline and acetylcholine, whereas deltamethrin potentiates the veratridine induced release. Therefore, although *N*-alkylamides and pyrethroids produce similar symptoms, they may act at different but possibly related sites of the membrane or Na⁺ channels.

If the pyrethroid/DDT resistance, which appears to be widespread and of major importance in pyrethroid-resistant arthropods,¹⁷⁾ is caused by a *kdr* mechanism as in houseflies, and shows comparable negative cross-resistance to *N*-alkylamides, this group may be a valuable source of commercial insecticides with the ability to counteract *kdr* by selecting against it.

Acknowledgments. The technical assistance of A. Peel and the financial support of the British Technology Group are gratefully acknowledged. U.K. and foreign patent

applications have been filed, based on U.K. patent application No. 8331036.

REFERENCES

- 1) M. Jacobson, "The Unsaturated Isobutylamides" in "Naturally Occurring Insecticides," ed. by M. Jacobson and D. G. Crosby, Dekker, New York, 1971, p. 137.
- 2) M. Miyakado, I. Nakayama, N. Ohno and H. Yoshioka, "Structure, Chemistry and Actions of the Piperaceae Amides: New Insecticidal Constituents Isolated from the Pepper Plant" in "Natural Products for Innovative Pest Management," ed. by D. L. Whitehead and W. S. Bowers, Pergamon, Oxford, 1983, p. 369.
- 3) M. Miyakado, I. Nakayama, A. Inoue, M. Hatakoshi and N. Ohno, *J. Pesticide Sci.*, **10**, 11 (1985).
- 4) M. Miyakado, I. Nakayama, A. Inoue, M. Hatakoshi and N. Ohno, *J. Pesticide Sci.*, **10**, 25 (1985).
- 5) M. Elliott, "Lipophilic Insect Control Agents" in "Recent Advances in the Chemistry of Insect Control," ed. by N. F. Janes, Royal Society of Chemistry, London, 1985, p. 73.
- 6) L. Crombie, *Nature*, **174**, 832 (1954).
- 7) M. Elliott and N. F. Janes, *Chem. Soc. Rev.*, **7**, 473 (1977).
- 8) M. Elliott, A. W. Farnham, N. F. Janes, D. M. Johnson and D. A. Pulman, *Pesticide Science* (7 papers in preparation or submitted for publication).
- 9) M. Elliott, A. W. Farnham, M. G. Ford, N. F. Janes and P. H. Needham, *Pesticide Science*, **3**, 25 (1972).
- 10) R. M. Sawicki, *Nature*, **875**, 4443 (1978).
- 11) C. W. Kearns and R. B. March, *Soap and Sanit. Chem.*, **19**, 101 (1943).
- 12) A. W. Farnham, *Pesticide Science*, **8**, 631 (1977).
- 13) M. P. Osborne and A. Smallcombe, "Site of Action of Pyrethroid Insecticides in Neural Membranes as Revealed by the *kdr* Resistance Factor" in "Pesticide Chemistry, Human Welfare and the Environment," Vol. 3, ed. by J. Miyamoto and P. C. Kearney, Pergamon, Oxford, 1983, p. 103.
- 14) R. W. Kerr, *C.S.I.R.O. Bulletin* (Australia), **261**, 30 (1951).
- 15) R. J. Blade, R. J. Hart and M. D. V. Moss, "Neuropharmacology and Pesticide Action," Abstracts of Neurotox 1985, Bath, 1985, p. 25; R. A. Nicholson, R. P. Botham and R. J. Blade, *ibid.*, p. 133.
- 16) D. W. Gammon, M. A. Brown and J. E. Casida, *Pestic. Biochem. Physiol.*, **15**, 181 (1981).
- 17) R. M. Sawicki, "Resistance to Pyrethroid Insecticides in Arthropods" in "Insecticides," ed. by D. H. Hutson and T. R. Roberts, Wiley, New York, 1985, p. 143.