ing binocular fixation can introduce shifts in visual direction that mimic the outcome expected with fusion [K. Ogle, *Researches in Binocular Vision* (Saunders, Philadelphia, 1950); Kaufman (*3*)]. This possibility can be minimized by using briefly flashed stimuli [Ono *et al.* (*7*)] or by measuring eye movements [Kertesz and Jones (*6*)]. A second, more general criticism of displacement phenomena concerns the potential ambiguities in criteria for reporting shifts in visual direction and in the sensitivity of the psychophysical procedures typically used [L. Kaufman and A. Arditi, *Vision Res.* **16**, 535 (1976)]. In general, these criticisms underscore the difficulty of distinguishing fusion from suppression on the basis of phenomenal report alone. To avoid these difficulties, others [R. Fox and R. Check, *Percept. Psychophys.* **1**, 331 (1966); R. Fox and C. McIntyre, *Psychon. Sci.* **8**, 143 (1967); W. Makous and R. K. Sanders, in *Visual Psychophysics: Its Physiological Basis*, J. Armington, J. Krauskopf, B. Wooten, Eds. (Erlbaum, Hillsdale, N.J., in press)] have used indirect techniques somewhat similar to ours, but the results have not been consistent.

6. A. Kertesz and R. W. Jones, *Vision Res.* **10**, 891 (1970).
7. H. Ono, R. Angus, P. Gregor, *Percept. Psychophys.* **21**, 513 (1977).
8. R. Fox and R. Check, *J. Exp. Psychol.* **78**, 388 (1968); R. Wales and R. Fox, *Percept. Psychophys.* **8**, 90 (1972); R. Blake and R. Fox, *Vision Res.* **14**, 687 (1974); R. Blake and S. Lema, *Vision Res.*, in press.
9. The test flash was generated by the driver and timing unit of a tachistoscope (Scientific Prototype model GB). The lamp was a cold-cathode fluorescent tube with a rise time to maximum intensity of approximately 1 msec. The position of the test flash within the left-eye display was controlled by a shutter. The center of the 10-minute circular test flash fell 15 minutes of arc from either the upper or lower edge of the left-eye target, depending on whether the flash was in the top or bottom position. Test flash luminance was 5.1 cd/m^2, and the circular spot was in cosine phase with respect to the sinusoidal striations of the grating, such that it covered one complete cycle of the grating. The observer viewed the entire display through natural pupils with the head firmly positioned on a dental impression board. A variable prism placed before one eye was used, if necessary, to achieve binocular alignment of the two CRT displays; the 1° circular aperture, which delimited the grating pattern, and the outer boundaries of the 8° by 10° CRT screen provided strong fusional stimuli for the maintenance of stable binocular alignment. Observers were instructed always to fixate the center of the circular aperture. The CRT screens provided the only illumination within the darkened test booth, and observers adapted to this prevailing level for at least 5 minutes before trials were begun. The position of the test flash was varied randomly from trial to trial under the control of a computer (PDP-8I), which also read and stored the observer's response on each trial. The results have been replicated upon repeated testing.
10. C. Blakemore, *Vision Res.* **10**, 1181 (1970); A. Fiorentini and L. Maffei, *ibid.* **11**, 1299 (1971); H. Wilson, *ibid.* **16**, 983 (1976).
11. We have also measured detection for the condition in which both eyes received uncontoured fields and the test probe went to the left eye. Performance was equivalent (about 90 percent) to that measured for conditions MD and BR-D, which indicates that the significant reduction in performance for condition MS, in which the left eye received an uncontoured field, arises from the contralateral pattern and not from fixation disparity, uncertainty about test flash location, or the absence of contour in the left eye.
12. For conditions BR-S and MS, the observer triggered test flash presentations while the grating viewed by the right eye was dominant. Although for conditions AF and ST, it would be impossible for observers to adopt this same strategy, on the basis of statistical considerations we would expect some decrement in performance even if suppression of the left eye were intermittent, as in the case of binocular rivalry. For each observer, when orthogonally oriented gratings were presented to the two eyes, the temporal pattern of binocular rivalry yielded approximately 50 percent predominance (percentage of time visible) for each eye, with average dominance durations on the order of 2 to 3 seconds. If we assume a comparable pattern of predominance during conditions AF and ST, detection performance for these conditions should be between 70 to 75 percent if suppression occurred intermittently. Performance was more accurate than this. It seems unlikely that a complete cycle of dominance and suppression could occur within the duration of the test flash, which ranged from 9.5 to 12 msec among observers. Moreover, we are confident these results are not due to eye dominance, since KB and PT are right-eye dominant while RB is left-eye dominant, as determined by conventional sighting tests [C. Porac and S. Coren, *Psychol. Bull.* **83**, 880 (1976)].
13. This locus of binocular fixation is on a plane known as the horopter and, according to the most accepted definition, is composed of those positions in visual space which appear to lie in the same direction in the visual field of both eyes [T. Shipley and S. Rawlings, *Vision Res.* **10**, 1225 (1970)].
14. We thank R. Sekuler for helpful comments. Supported by grant BNS77-15858 from the National Service Foundation.

7 October 1977; revised 8 February 1978

Insecticidal Benzoylphenyl Ureas: Structure-Activity Relationships as Chitin Synthesis Inhibitors

Abstract. *The 1-benzoyl-3-phenylurea insecticide diflubenzuron is a potent inhibitor for the conversion of* ^{14}*C-labeled glucose to* ^{14}*C-labeled chitin in isolated abdomens of newly emerged adult milkweed bugs* (Oncopeltus fasciatus *Dallas). The inhibitory activity of 24 diflubenzuron analogs in this in vitro chitin-synthesizing system is in good agreement with their toxicity to fifth instar nymphs of this species. These insecticides act quickly and directly within the integument to ultimately block the terminal polymerization step in chitin formation.*

Chitin is the most abundant organic skeletal component of insects, other invertebrates, and many fungi, but it is absent in vertebrates and higher plants (*1*). Insecticides that disrupt chitin deposition therefore have selectivity advantages over earlier types that alter nerve action or bioenergetic reactions that are similar in insects and mammals. Diflubenzuron [2,6-F_2-C_6H_3-C(O)NHC(O)-NH-C_6H_4-Cl-4] and several other benzoylphenyl ureas effectively control major insect pests by interfering with the molting process or by acting as ovicides and chemosterilants (*2*).The larvicidal activity is attributable to disruption of chitin deposition (*2*). This benzoylphenyl urea action may be indirect by altering ecdysone or juvenile hormone levels (*3*) or direct by inhibiting a critical step in chitin formation (*4*). An insect system for in vitro chitin biosynthesis is required to differentiate between these hypotheses. We find, using abdomens of newly emerged adult milkweed bugs (*Oncopeltus fasciatus* Dallas) as reaction vessels, that benzoylphenyl ureas act directly within the integument to block the terminal polymerization step in chitin formation (*5*).

An ideal insect system for studies on inhibitors of chitin biosynthesis should meet the following specifications: rapid and consistent in vitro formation of ^{14}C-labeled chitin in reasonable yields from convenient ^{14}C precursors such as glucose, glucosamine, and *N*-acetylglucosamine; sensitivity to polyoxin D, a chitin synthetase inhibitor (*6*), and to diflubenzuron and its insecticidal analogs; and no involvement of exogenous hormones during the period of insecticide action. Cultures of cockroach leg regenerates meet some of these requirements, but this system requires activation by exogenous β-ecdysone and an assay period of 2 weeks (*7*). In developing our system, we used milkweed bugs for several reasons. They are easy to rear and handle, and the fifth instar nymphs are highly sensitive to topically applied diflubenzuron and its analogs (*8*). Insecticidal levels of diflubenzuron do not alter the in vivo metabolic conversion of α-ecdysone to β-ecdysone or the subsequent metabolism of β-ecdysone in fifth instar milkweed bug nymphs (*5*) or the endogenous β-ecdysone titers in pharate pupae of *Stomoxys calcitrans* whose larvae had been exposed to the insecticide (*9*). Although these findings tend to rule out hormone mediation in the action of diflubenzuron, we chose to further minimize the possibility of hormone effects by using young adults since their endogenous ecdysone levels are low (*10*); in immature insects the β-ecdysone titers reach maximum levels shortly before molting (*10*) and strongly influence chitin biosynthesis (*11*).

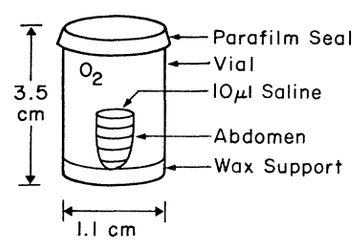

Fig. 1. Diagram of the isolated adult milkweed bug abdomen used as reaction vessel for chitin biosynthesis.

Milkweed bug adults were used 12 hours after emergence because at this time their activity for converting [^{14}C]glucose to [^{14}C]chitin is higher than it is either earlier or later. The abdomen

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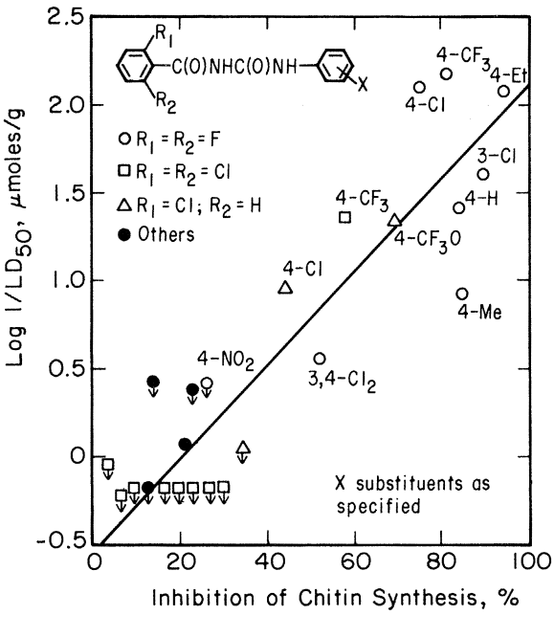

Fig. 2. Relation between the activity of 24 benzoylphenyl ureas and related compounds at $3 \times 10^{-6}M$ as inhibitors of [^{14}C]chitin biosynthesis from [^{14}C]glucose in the adult *O. fasciatus* in vitro abdomen system and their toxicity to fifth instar *O. fasciatus* nymphs. Points with a vertical arrow designate compounds that are inactive at 500 μg per gram of insect body weight or at the maximum amounts that could be applied to the insects, with acetone as the carrier vehicle.

was removed from the insect, the contents were pulled out, and the resulting tube was supported in wax in a small vial (Fig. 1) (*12*). A microsyringe was used to charge this reaction vessel first with insect saline (10 μl) (*13*) containing the test insecticide in a final concentration of 0.2 percent dimethylsulfoxide, and then with [^{14}C]glucose (0.4 μg in 1 μl saline) or other labeled precursor. The vial was flushed with oxygen and sealed with Parafilm. At various times thereafter the abdomens were removed, and the ^{14}C content of the chitin was determined (*14*).

[^{14}C]Chitin synthesis from [^{14}C]glucose proceeded rapidly for 1 hour and [^{14}C]glucosamine and *N*-[^{14}C]acetylglucosamine are also suitable precursors, each giving about 3 to 5 percent incorporation. Polyoxin D inhibits [^{14}C]-chitin formation by 50 percent when added at $1.2 \times 10^{-5}M$ along with [^{14}C]glucose. Diflubenzuron is 22 times more potent, inhibiting 50 percent at $5.5 \times 10^{-7}M$ either on simultaneous addition of diflubenzuron and [^{14}C]glucose or on addition of the insecticide 30 minutes prior to treatment with [^{14}C]glucose. Inhibition by diflubenzuron is therefore very quick, and the effective dose (0.25 μg per gram of abdomen) was only one-tenth that required to kill fifth instar nymphs (*8*). With *N*-[^{14}C]acetylglucosamine as the substrate and a diflubenzuron level that inhibits chitin formation by 43 percent, this insecticide did not affect transport of the substrate or its metabolites into the integument but within 20 minutes it produced a 32 percent increase in the amount of uridine 5′-diphospho-*N*-[^{14}C]acetylglucosamine within the integument. These quantitative results and preliminary qualitative findings with other in vitro and in vivo insect systems (*4*) indicate that diflubenzuron ultimately blocks the terminal polymerization step in chitin formation.

Comparison of 24 diflubenzuron analogs (*15*) revealed a good correlation (*16*) between inhibition of chitin synthesis in the adult abdomen in vitro system and toxicity to fifth instar milkweed bug nymphs (Fig. 2). Particularly potent compounds are diflubenzuron and its 2,6-difluorobenzoyl analogs (except the 4-nitro derivative), the 2-chlorobenzoyl compounds with 4-trifluoromethoxy and 4-chloro substituents, and the 2,6-dichlorobenzoyl analog with a 4-trifluoromethyl substituent.

The correlation of chitin synthesis inhibition with insecticidal potency has several important implications. The insecticidal action of the benzoylphenyl ureas results from direct inhibition of chitin synthesis within the integument rather than from any indirect extracuticular effects on hormone levels. The site of action (receptor) for diflubenzuron in the integument is probably similar in nymphs and adults. The receptor conformation may be generally more important than differences in penetration, distribution, and metabolism in determining the optimal structures for high potency. There are large species variations in the relative potencies of various benzoylphenyl ureas (*17*) and therefore the conformation of the receptor may vary in different species.

The adult milkweed bug in vitro abdomen system for investigations on chitin biosynthesis should be useful in further optimizing the structure and defining the molecular basis for the action of benzoylphenyl ureas and related insecticides.

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References and Notes

1. A. C. Neville, *Biology of the Arthropod Cuticle* (Springer-Verlag, New York, 1975); H. R. Hepburn, Ed., *The Insect Integument* (Elsevier, New York, 1976).
2. A. Verloop and C. D. Ferrell, *Am. Chem. Soc. Symp. Ser.* **37** (1977), pp. 237–270.
3. S. J. Yu and L. C. Terriere, *Life Sci.* **17**, 619 (1975); *Pestic. Biochem. Physiol.* 7, 48 (1977).
4. L. C. Post, B. J. de Jong, W. R. Vincent, *Pestic. Biochem. Physiol.* **4**, 473 (1974); E. P. Marks and B. A. Sowa, in *The Insect Integument*, H. R. Hepburn, Ed. (Elsevier, New York, 1976), pp. 339–357.
5. N. P. Hajjar and J. E. Casida, paper 27 of Pesticide Chemistry Division, 173rd National Meeting, American Chemical Society, New Orleans, La., 21 to 25 March 1977.
6. M. Hori, K. Kakiki, T. Misato, *Agr. Biol. Chem.* **38**, 691 (1974); T. Misato, *Am. Chem. Soc. Symp. Ser.* **37** (1977), pp. 170–192.
7. B. A. Sowa and E. P. Marks, *Insect Biochem.* **5**, 855 (1975). Comparable exogenous β-ecdysone-dependent systems with wing or leg imaginal disks include those of *Drosophila melanogaster* (Diptera) [P. Mandaron, *Wilhelm Roux' Arch. Dev. Biol.* **179**, 185 (1976)], *Chilo suppressalis* (Lepidoptera) [N. Agui, S. Yagi, M. Fukaya, *Appl. Entomol. Zool.* **4**, 158 (1969)], and *Plodia interpunctella* (Lepidoptera) (H. Oberlander and C. E. Leach, in *Proceedings of the First International Working Conference on Stored-Product Entomology, Savannah, Ga., 1974*). Diflubenzuron was tested in the *P. interpunctella* system only and did not inhibit chitin synthesis.
8. The lethal dose for 50 percent of the nymphs (LD_{50}) was 2.4 μg of diflubenzuron per gram of insect body weight. With this and other benzoylphenyl ureas, the insects generally die during the molt subsequent to treatment.
9. M. P. O'Neill, G. M. Holman, J. E. Wright, *J. Insect Physiol.* **23**, 1243 (1977).
10. D. Feir and G. Winkler, *ibid.* **15**, 899 (1969); P. Karlson, J. Koolman, J. A. Hoffmann, *Am. Zool.* **15** (Suppl. 1), 49 (1975).
11. S. Kimura, *J. Insect Physiol.* **19**, 2177 (1973); M. Locke, in *Insect Integument*, H. R. Hepburn, Ed. (Elsevier, New York, 1976), pp. 237–258.
12. Comparable systems include *Locusta migratoria* (Orthoptera) [B. Surholt, *J. Comp. Physiol.* **102**, 135 (1975)] and *Melanoplus sanguinipes* (Orthoptera) [A. Vardanis, *Life Sci.* **19**, 1949 (1976)]. The action of benzoylphenyl ureas in these systems is not reported.
13. The saline consists of the following salts (grams per liter): NaCl, 9; KCl, 0.8; $CaCl_2$, 0.44; $NaH_2PO_4 \cdot 2H_2O$, 0.2; $MgCl_2 \cdot 6H_2O$, 0.1; and $NaHCO_3$, 0.05.
14. I. Ishaaya and J. E. Casida, *Pestic. Biochem. Physiol.* **4**, 484 (1974). The described method was modified by adding 50 mg of unlabeled chitin before extraction, purification by treatment with 10 percent NaOH at 100°C and 2 percent HCl at 25°C, and determination of ^{14}C incorporation by combustion and liquid scintillation counting.
15. Kindly provided by C.-C. Yu and R. J. Kuhr [*J. Agric. Food Chem.* **24**, 134 (1976)]; Thompson-Hayward Chemical Co. (Kansas City, Kan.); Mobay Chemical Corp. (Vero Beach, Fla.); Eli Lilly and Co. (Indianapolis, Ind.).
16. Log $1/LD_{50} = -0.274 + 0.0234$ (inhibition, percent); $n = 11$, $\bar{R}^2 = .574$, $P < .01$; where n is the number of replicates, $\bar{R}^2$ is the adjusted correlation coefficient, and P is the probability. These calculations utilize only the 11 compounds that are sufficiently toxic for LD_{50} determinations. The correlation is further supported by the finding that no one of the 13 remaining low toxicity compounds is a potent chitin synthesis inhibitor (see Fig. 2).
17. K. Wellinga, R. Mulder, J. J. van Daalen, *J. Agric. Food Chem.* **21**, 348 and 993 (1973).
18. Supported in part by NIH grant 5 P01 ES00049.

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