TOXICOLOGICAL AND BIOCHEMICAL ASPECTS OF NOVEL ACYLUREAS ON RESISTANT AND SUSCEPTIBLE STRAINS OF *TRIBOLIUM CASTANEUM*

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Abstract

The novel acylureas XRD-473, CME-134 and IKI-7899 were 4- to 23-fold more toxic than diflubenzuron and exhibited similar toxicity on both malathion-susceptible (bb) and -resistant (CTC-12) strains of Tribolium castaneum. On the other hand, diflubenzuron was considerably less toxic to the resistant strain. When larval weight inhibition (at 14 days after start of treatment) was determined, the potency of the new compounds relative to diflubenzuron was over 20- and 150-fold at IC-50 and IC-95, respectively. According to mortality curves and LC-50 values the toxicity of the test compounds on both Tribolium strains was XRD-473 > CME-134 $\geq$ IKI-7899 > diflubenzuron.

With all the new acylureas (but not with diflubenzuron), a considerable number of pupa-adult intermediates and abnormal adults was observed. These aberrations resulted probably from the stability and persistence of the new compounds during the larval and pupal stages.

The high potency of XRD-473, CME-134 and IKI-7899 on both malathion-susceptible and -resistant strains of Tribolium, along with their low mammalian toxicity, render these compounds potential insect control agents, which should be tested further for their safety in the environment and for their possible use against stored-product pests.

Introduction

Acylureas are selective insecticides acting on insects of various orders by inhibiting chitin formation (Ishaaya and Casida, 1974; Post et al., 1974), thereby causing abnormal endocuticular deposition and abortive molting (Mulder and Gijswijt, 1973). Diflubenzuron, the most investigated compound of this series, affects the postapolytic stage of larvae (Grosscurt, 1978) and in some species suppresses fecundity (Arambourg et al., 1977; Sarasua and Santiago-Alvarez, 1983) and exhibits ovicidal and contact toxicity (Ascher and Nemny, 1974,

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1976; Holst, 1975; Wright and Harris, 1976). Treated larvae usually feed normally until the apolytic stage and may skip, in some cases, two or three molts. Hence, some economically important agricultural pests, such as Spodoptera and Heliothis species, cannot be controlled efficiently under field conditions (Grosscurt, 1978). The continuous search for more potent acylureas has led recently to the development of new compounds such as IKI-7899 (chlorfluazuron, Ishihara Sangyo Kaisha Ltd.), CME-134 (teflubenzuron, Celamerck GmbH & Co.) and XRD-473 (Dow Chemicals) (Fig. 1), which are considerably more potent than diflubenzuron on various agricultural pests (Haga et al., 1982; Becher et al., 1983; Sbragia et al., 1983).

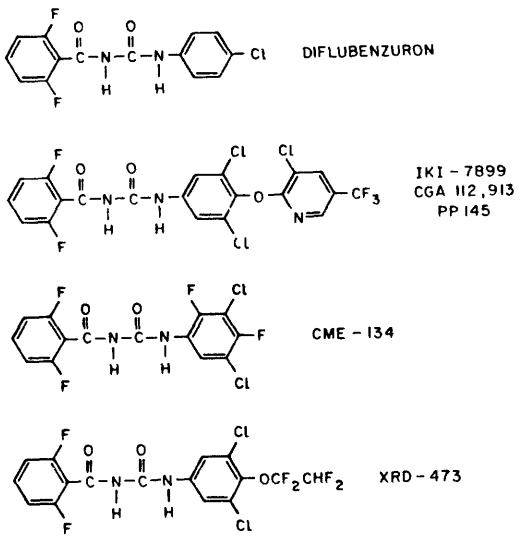

Fig. 1. Structure of the acylurea compounds.

IKI-7899 has been shown to persist much longer than the early benzoylphenylurea diflubenzuron in Spodoptera littoralis and thus to block chitin biosynthesis much more effectively (Neumann and Guyer, 1983). In fact, IKI-7899 is five-fold more toxic than diflubenzuron on topical application to S. littoralis larvae (Ishaaya et al., 1984). CME-134 is much more potent than diflubenzuron and triflumuron against Spodoptera larvae in the laboratory and extremely more persistent than diflubenzuron in the field (Ascher and Nemny, 1984); the same is true for Prodenia eridania and several other agricultural pests (Becher et al., 1983). XRD-473 has a broader biological activity spectrum than the earlier benzoylphenylureas and is faster acting, exhibiting also some conventional-type toxicity (Sbragia et al., 1983).

An imminent danger to any novel insect control agent is the development of resistant strains, in particular in species that have developed resistance to the commonly used insecticides. Strains of the house fly, Musca domestica L., that are resistant to conventional insecticides, have been shown to be variously cross-resistant to diflubenzuron (Cerf and Georghiou, 1974; Oppenoorth and Van Der Pas, 1977; Pimprikar and Georghiou, 1979). On the other hand, IKI-7899 exhibits equal toxicity to malathion-resistant and -susceptible strains of Tribolium castaneum (Ishaaya and Yablonski, 1986). The present study evaluates toxicity and biochemical aspects of IKI-7899, CME-134 and XRD-473 in comparison with those of diflubenzuron on malathion-susceptible and -resistant strains of Tribolium castaneum.

Materials and Methods

Chemicals. Technical acylureas were supplied from the indicated sources: diflubenzuron from Duphar B.V. ('s-Graveland, Holland), IKI-7899 from Ishihara Sangyo Kaisha Ltd. (Kusatsu, Japan), CME-134 from Celamerck GmbH & Co. (Ingelheim, W. Germany), and XRD-473 from Dow Chemicals (Michigan, USA).

Rearing and Assays. Two strains of Tribolium castaneum were used: one malathion-resistant (CTC-12) and the other malathion-susceptible (bb). Biochemical and genetic determination (Cohen, 1981; Wool et al., 1982) indicated that the CTC-12 strain exhibited higher detoxifying enzymes of about four-fold more activity of cytochrome P-450 and of epoxide hydrase than the bb strain.

The rearing procedure, using wheat flour containing 5% dried yeast as the basic diet, and the bioassay method were the same as described previously (Ishaaya and Ascher, 1977; Ishaaya et al., 1981). For bioassays, the diet (10 g) was mixed with acetone solution (10 ml) containing the test compound, or with acetone (10 ml) alone as the control. The diet was distributed in 2-g portions in test vials (2 cm in diameter) following thorough mixing and solvent evaporation. Each treatment was carried out with 15-20 replicates of 15 first-instar larvae and held at 28 C for determination of larval mortality, pupation and emergence. For comparison, Probit/log concentration lines were used to determine LC-50 and LC-95 (concentrations needed for 50 and 95% cumulative larval and pupal mortality) or IC-50 and IC-95 (concentrations needed for 50 and 95% larval weight gain inhibition at day 14) values for the various chitin synthesis inhibitors. Delayed effects, as expressed by pupa-adult intermediates and abnormal adults were determined according to Ishaaya (1982).

Results and Discussion

According to LC-50 and LC-95 values, XRD-473, CME-134 and IKI-7899 were 4- to 23-fold more toxic than diflubenzuron and exhibited similar toxicity on both malathion-susceptible (bb) and -resistant (CTC-12) strains of Tribolium castaneum (Fig. 2, Table 1). On the other hand, diflubenzuron was 1.5- and 2.5-fold less toxic to the resistant strain according to LC-50 and LC-95 values, respectively, as compared with the susceptible strain (Table 1), which seems to be due to diflubenzuron's susceptibility to the relatively high oxidative and hydrolytic activities present in the resistant strain (Wool et al., 1982). Accordingly, the potency of these compounds relative to diflubenzuron increased considerably in the CTC-12 strain, reaching ratios of 14- to 23-fold at LC-95 (Table 1). This phenomenon was even more conspicuous when larval weight gain inhibition at 14 days after start of treatment was determined (Fig. 3, Table 2). In these assays, the potency of the new acylureas on the CTC-12 strain was over 20-fold relative to diflubenzuron at IC-50 and over 150-fold at IC-95. According to mortality curves and LC-50 values, the relative toxicity of the test compounds on both Tribolium strains was XRD-473 > CME-134 ≥ IKI-7899 > diflubenzuron.

Table 1. LC-50 and LC-95 values in $mgkg^{-1}$ and (potency relative to diflubenzuron, in parentheses) of four chitin synthesis inhibitors obtained with malathion-susceptible (bb) and -resistant (CTC-12) strains of Tribolium castaneum. Data are mean of 15-20 replicates of 15 first-instar larvae each. Cumulative larval and pupal mortality was determined.

Compounds	bb	CTC-12
	LC-50	
XRD-473	0.068a* (7.1)	0.070a (10.2)
CME-134	0.092b (5.2)	0.104b (6.9)
IKI-7899	0.106b (4.5)	0.108b (6.7)
Diflubenzuron	0.480c	0.720c
	LC-95	
XRD-473	0.108a (9.8)	0.116a (22.6)
CME-134	0.148b (7.2)	0.188b (14.0)
IKI-7899	0.156b (6.8)	0.176b (14.9)
Diflubenzuron	1.060c	2.63c

*Data followed by different letters are significantly different from each other at P = 0.05 within the same group and column.

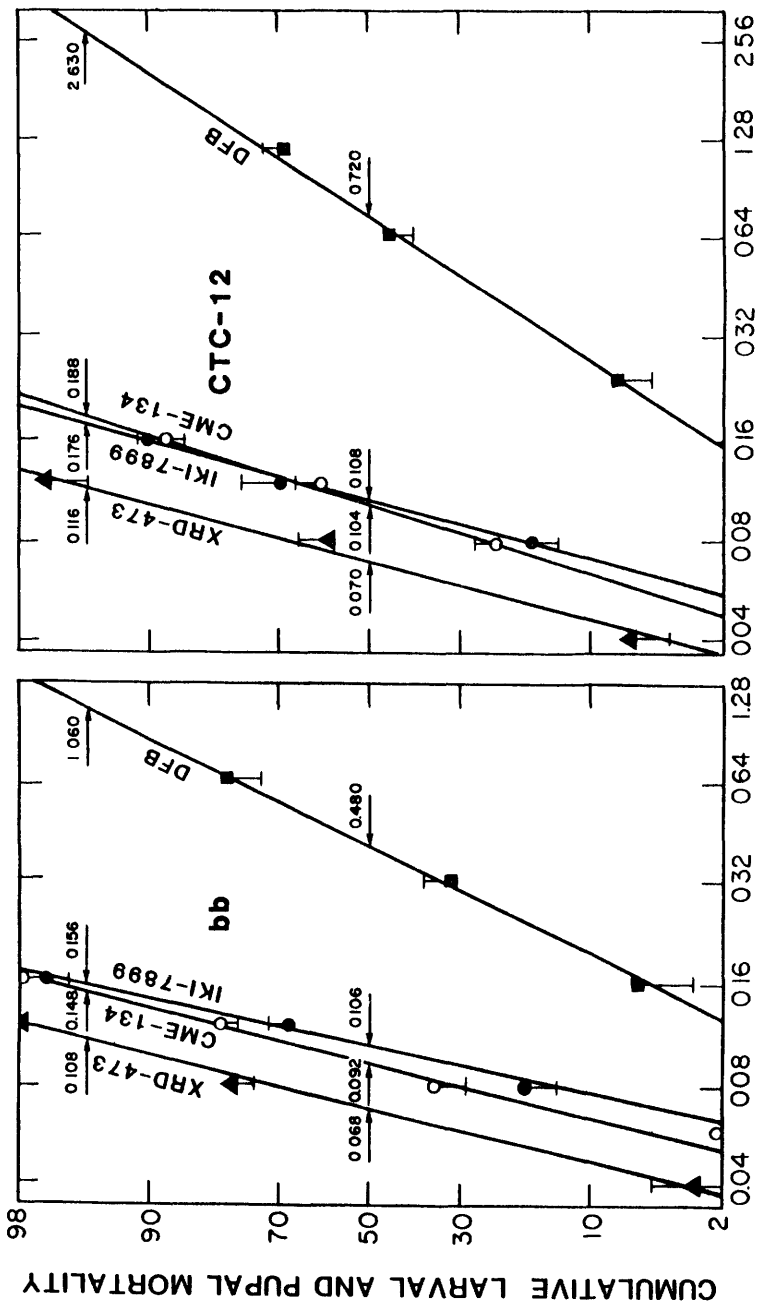

Fig. 2. Log dosage-percentage mortality curves on a probit scale for dietary applied XRD-473, CME-134, IKI-7899 and diflubenzuron (DFB) on malathion-susceptible (bb) and -resistant strains (CTC-12). The arrows designate LC-50 and LC-95 values.

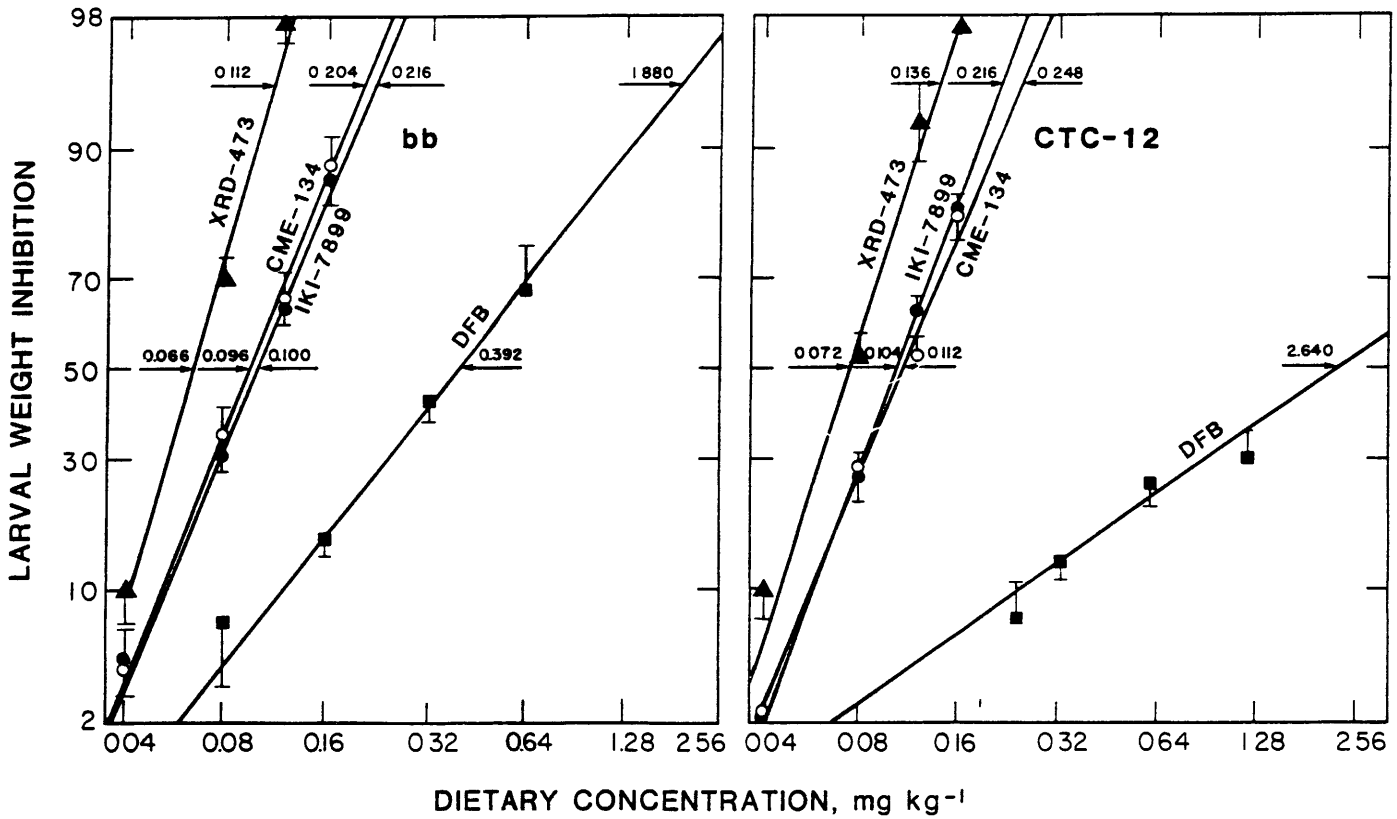

Fig. 3. Log dosage-percentage weight gain inhibition curves on a probit scale for dietary applied XRD-473, CME-134, IKI-7899 and diflubenzuron (DFB) on malathion-susceptible (bb) and -resistant strains (CTC-12). The arrows designate 50% (IC-50) and 95% (IC-95) weight gain inhibition.

Table 2. Effect of four chitin synthesis inhibitors on larval weight gain of malathion-susceptible (bb) and -resistant (CTC-12) strains of T. castaneum. Weight gain inhibition at 50% (IC-50) and 95% (IC-95) levels in $mgkg^{-1}$ and (potency relative to diflubenzuron, in parentheses) of four chitin was determined on day 14. Data are means of 15-20 replicates of 15 first-instar larvae each.

Compounds	bb	CTC-12
	IC-50	
XRD-473	0.066a* (5.9)	0.072a (36.7)
CME-134	0.096b (4.1)	0.112b (23.6)
IKI-7899	0.100b (3.9)	0.104b (25.4)
Diflubenzuron	0.392c	2.640c
	IC-95	
XRD-473	0.112a (16.8)	0.136a (301)
CME-134	0.204b (9.2)	0.216b (190)
IKI-7899	0.216b (8.7)	0.248b (165)
Diflubenzuron	1.880c	41.000c

*Data followed by different lettters differ significantly from each other at P = 0.05 within the same group and column.

A considerable number of pupa-adult intermediates and abnormal adults was observed in all assays with XRD-473, CME-134 and IKI-7899, but not with diflubenzuron (Tables 3 and 4). These aberrations may result from the stability of the new acylureas to metabolic detoxification during the larval and pupal stages, resulting thereby in deformation of pupae and adults. These findings concur with those obtained by Neumann and Guyer (1983), who showed that diflubenzuron is more susceptible than IKI-7899 to detoxification in Spodoptera littoralis larvae.

Our results and those reported by others (Cerf and Georghiou, 1974; Oppenoorth and Van Der Pas, 1977; Pimprikar and Georghiou, 1979) strongly indicate the possibility of cross resistance between organophosphorus insecticides and diflubenzuron. On the other hand, the novel benzoylphenylureas (IKI-7899, CME-134 and XRD-473) seem to have a relatively high stability to detoxifying enzymes in the insects and as such, each of them shows similar potency on malathion-susceptible and -resistant strains.

The high potency and prolonged stability of IKI-7899, CME-134 and XRD-473 in malathion-susceptible and -resistant strains of Tribolium and in other agricultural pests, along with their low toxicity to man, predators and parasites, render these compounds potential insect control agents to be tested in integrated pest management programs for controlling stored-product pests and especially those resistant to malathion and other organophosphorus compounds.

Table 3. Effect of four chitin synthesis inhibitors on the growth and development of the malathion-susceptible strain of T. castaneum (bb)*. PA = pupa-adult intermediates; AA = abnormal adults.

Dietary concentration, $mgkg^{-1}$	Larval mortality, %	PA+AA, %	Normal adults, %
Diflubenzuron			
0.48	63±3a**	0	37±3a
0.64	80±6b	0	20±6b
IKI-7899			
0.12	77±4a	7±3	16±3a
0.16	85±4b	4±1	11±4a
CME-134			
0.12	64±7a	25±4	11±4a
0.16	95±2b	4±2	1±1b
XRD-473			
0.08	74±5a	14±5	12±3a
0.12	100b	0	0b

*Data are means ± SE values of 15-20 replicates of 15 first-instar (0-3 h old) larvae each.
**Data followed by different letters are significantly different at P = 0.05 within the same compound and column.

Table 4. Effect of four chitin synthesis inhibitors on the growth and development of a malathion-resistant strain of T. castaneum (CTC-12)*. PA = pupa-adult intermediates; AA = abnormal adults.

Dietary concentration, $mgkg^{-1}$	Larval mortality, %	PA+AA, %	Normal adults, %
Diflubenzuron			
0.64	47±4a**	0	53±4a
1.28	69±4b	0	31±4b
IKI-7899			
0.12	63±7a	8±1	29±6a
0.16	83±3b	8±2	4±1b
CME-134			
0.12	48±8a	20±4	32±5a
0.16	80±6b	9±2	11±4b
XRD-473			
0.08	41±8a	27±6	32±6a
0.12	95±3b	2±2	3±2b
0.16	100b	0	0b

*Data are means ± SE values of 15-20 replicates of 15 first-instar (0-3 h old) larvae each.
**Data followed by different letters are significantly different from each other at P=0.05 within the same compound and column.

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