Heterocyclic Analogues of Diflubenzuron as Growth and Reproduction Inhibitors of the Fall Armyworm and House Fly

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Twenty-six new analogues of diflubenzuron were synthesized and tested as inhibitors of growth and reproduction for the fall armyworm and house fly. The most effective growth inhibitors for the fall armyworm were *N*-[[(5-chloro-2-pyridinyl)amino]carbonyl]-2,6-difluorobenzamide (**11**) and *N*-[[(5-bromo-2-pyridinyl)amino]carbonyl]-2,6-difluorobenzamide (**12**), both with activity comparable to that of diflubenzuron. Compounds **11** and **12** were also the most effective for inhibiting egg hatch when injected into adult house flies. The most effective growth inhibitors for the house fly were compound **12**, *N*-[[(6-chloro-3-pyridinyl)amino]carbonyl]-2,6-difluorobenzamide (**17**), and 2,6-difluoro-*N*-[[(4-phenyl-2-thiazolyl)amino]carbonyl]benzamide (**6**), but all three were less effective than diflubenzuron. Of all heterocyclic analogues tested the pyridine derivatives gave the best overall performance as inhibitors of growth and reproduction.

Diflubenzuron (*N*-[[(4-chlorophenyl)amino]carbonyl]-2,6-difluorobenzamide (**1**) is a potent chitin inhibitor reported to control a wide variety of insect pests (Wellinga et al., 1973a, Technical Bulletin "TH-6040—Insect Growth Regulator", Thompson-Hayward Chem. Co., 1974). Considerable effort has been applied to develop new and more efficacious compounds related to this pesticide, but only three reports have appeared describing heterocyclic analogues of diflubenzuron or its dichloro analogue TH-6038 (**2**) (*N*-[[(4-chlorophenyl)amino]carbonyl]-2,6-dichlorobenzamide). These describe heterocycles formed by (a) ring closure of the two urea nitrogens of TH-6038 (Wellinga et al., 1973b), (b) fusion of the urea oxygen of **1** to the benzene ring of the aniline moiety (Oliver et al., 1976), and (c) replacement of the *p*-chloroaniline moiety of **2** by substituted aminopyrazines (Miesel, 1976). To extend the work of Miesel we have synthesized 26 new heterocyclic analogues of diflubenzuron and report here their growth- and reproduction-inhibiting effects in the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) and the house fly, *Musca domestica* L.

MATERIALS AND METHODS

Syntheses of Chemicals. Melting points are uncorrected. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. All compounds except **1** and **2** are new and gave acceptable analyses for C and H. Tables of complete analytical data for the compounds will appear in the microfilm edition (see paragraph at end of paper regarding supplementary materials). The heterocyclic derivatives were prepared by the condensation of the appropriate amino-substituted heterocycle with 2,6-difluorobenzoyl isocyanate in acetonitrile. 2,6-Difluorobenzoyl isocyanate, bp 85 °C (10 mm), was prepared by treating 2,6-difluorobenzamide (obtained by H_2SO_4 hydrolysis of the corresponding nitrile, Wellinga et al., 1973a) with oxalyl chloride by a procedure similar to that used for the preparation of its dichloro analogue (Wellinga et al., 1973b).

All compounds listed in Table I were assigned urea structures that correspond to a normal addition of exocyclic nitrogen of the heterocycle to the benzoyl isocyanate. These assignments were made on the basis of products described in the literature for analogous reactions involving addition of amino-substituted heterocycles (similar to the ones used in our study) to various aryl and alkyl isocyanates or isothiocyanates.

Biological Tests. The fall armyworm larval tests were conducted as follows: Test compounds were incorporated into 100 g of standard diet (Redfern and Raulston, 1970) at the rate of 100, 50, 10, 1, 0.1, or 0.01 ppm. Treated diets were poured into 1-oz cups (8 g/cup). One third or fourth instar larva was introduced in each cup (ten larvae used for each concentration), and the cups were held at 27 ± 1 °C (RH 50 ± 5%) and observed daily for dead larvae. Larvae that survived treatment were observed for adult emergence. The effectiveness of a compound was assessed on the basis of percentage of adult emergence inhibition, and the following scale of graded activity was used: 0, no inhibition at 100 ppm; +, less than 80% inhibition at 100 ppm; ++, 80–100% inhibition at 100 ppm; +++, 80–100% inhibition at 10 ppm; ++++, 80–100% inhibition at 1 ppm; +++++, 80–100% inhibition at 0.1 ppm. Control values averaged 5% adult emergence inhibition, and two replicates were used for each concentration.

Sterility in fall armyworms was determined by allowing the adults surviving the larvicidal treatment to mate and then comparing the viability and quantity of eggs produced by the treated insects to those of the controls.

In the house fly larval test, 50 eggs were placed in a covered petri dish to which was added the larval medium (ca. 20 mL of a 1% agar jell, containing 10% of a mixture of pulverized hog chow, yeast, and powdered liver (1:1:2)) and the test compounds at 10, 1, 0.1, or 0.01 ppm concentration. The larvae were allowed to pupate and the pupae were held for adult emergence. The percentage of eggs that survived to the adult stage was used to measure the efficacy of the compound.

Sterility in adult house flies was determined by administering the compounds orally or by injection. In the injection method 10 μg of the test compound, dissolved in 0.208 μL of a 1:1 (v/v) dimethyl sulfoxide–acetone solution, was injected into each female fly. Sterility was determined by allowing ten treated females to mate with ten untreated males, collecting the eggs, and scoring them for hatch as described by Chang et al. (1970).

Details of the feeding method were previously described (Fye et al., 1966). Briefly, each compound was added to a diet of sucrose, nonfat dry milk, and powdered egg yolk

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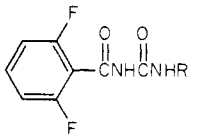
Table I. Test Results of Heterocyclic Analogues of Diflubenzuron in the Fall Armyworm and House Fly



No.	ARS No.	R	Mp, °C	Fall armyworm larvicidal activity[a]	House fly sterility, %[b] (injected dose of 10 μg/female)
1	29054	4-Chlorophenyl	c	+++++	100
2	29053[d]		c	++++	19
3	63358	3,4-Dimethyl-5-isoxazolyl	205-208	+++	4
4	63364	4-Chloro-5-methyl-3-isoxazolyl	207-210	++	e
5	63334	5-Chloro-2-thiazolyl	214-217	++++	34
6	63310	4-Phenyl-2-thiazolyl	248-251	++	18
7	63339	5-(1-Methylethyl)-1,3,4-thiadiazol-2-yl	206	++	15
8	63338	5-(Trifluoromethyl)-1,3,4-thiadiazol-2-yl	212-214 dec	++	
9	63363	5-(Methylthio)-1,3,4-thiadiazol-2-yl	185-187	0	e
10	63351	3-Methyl-1,2,4-thiadiazol-5-yl	230 dec	++	e
11	63308	5-Chloro-2-pyridinyl	222-223	+++++	71
12	63354	5-Bromo-2-pyridinyl	217-219	+++++	75
13	63355	5-Nitro-2-pyridinyl	214-216	+++	10
14	63398	5-Methyl-2-pyridinyl	199-201.5	++++	9
15	63356	3,5-Dichloro-2-pyridinyl	188-192	+++	e
16	63357	3,5-Dibromo-2-pyridinyl	184-192	++	e
17	63307	6-Chloro-3-pyridinyl	233-235	++++	14
18	63335	4-Chloro-6-methyl-2-pyrimidinyl	183.5-184.5	0	36
19	63336	6-Chloro-2-(methylthio)-4-pyrimidinyl	234-234.5	0	7
20	63352	4,6-Bis(dimethylamino)-1,3,5-triazin-2-yl	218-219	+	e
21	63353	4,6-Dimethoxy-1,3,5-triazin-2-yl	155-157	+	0
22	63304	2-Benzoxazolyl	187-187.5	++	34
23	63312	5-Chloro-2-benzoxazolyl	201 dec	0	9
24	63305	2-Benzothiazolyl	240 dec	++	14
25	63309	4-Chloro-2-benzothiazolyl	244-245	++	19
26	63337	5-Chloro-1*H*-benzimidazol-2-yl	>300	+++	1
27	63311	1-*H*-Indol-5-yl	207-209	++	13
28	63349	2-Methyl-6-benzothiazolyl	208-210	+++	e

[a] See Materials and Methods for key to graded activities. [b] Determined by egg hatch. Sterility of controls <5%. [c] Wellinga et al. (1973a). [d] TH-6038. [e] 100% mortality at 10 μg with less than 25% sterility at sublethal concentrations.

(6:6:1 w/w). Adult flies of both sexes kept on the treated diet for 7 days were allowed to mate, and their reproductive performance was evaluated and compared with that of control flies. Sterility was assessed from the percentage of eggs that developed into pupae.

RESULTS AND DISCUSSION

Results from structure–activity relationship studies (Wellinga et al., 1973a,b) indicated that slight variation from the basic 2,6-dihalo configuration in the benzoyl moiety of diflubenzuron decreased activity substantially whereas position and/or substituent changes in the aniline moiety of the molecule had a less severe effect on activity. Taking this into account we prepared a number of analogues of diflubenzuron wherein we replaced the benzene ring in the aniline portion of the molecule with various substituted heterocycles. We selected five-membered rings such as isoxazoles (**3**, **4**), thiazoles (**5**, **6**), and thiadiazoles (**7**–**10**), six-membered rings such as pyridines (**11**–**17**), pyrimidines (**18**, **19**), and *s*-triazines (**20**, **21**) and various bicyclic heterocycles (**22**–**28**) (Table I).

Results from fall armyworm larval growth tests showed the 5-bromo- and 5-chloro-2-pyridinyl analogues **12** and **11** (approximately isosteric with diflubenzuron) were the most effective compounds with activities comparable to that of diflubenzuron. Compound **17**, another approximate isostere of diflubenzuron containing a 6-chloro-3-pyridinyl moiety, was less effective than its 2-pyridinyl isomer, compound **11**. With an occasional exception, substitution of the benzene ring at the ortho position with halogen substituents lowered activity (Wellinga et al., 1973a,b). This effect was also observed for the dihalogen-substituted compounds **15** and **16**, which were less active than their respective monosubstituted analogues **11** and **12**. Other nonpyridinyl analogues causing substantial inhibition of growth in fall armyworms were **3**, **5**, **26**, and **28**. The thiazole derivative **5**, while less effective than diflubenzuron, had larvicidal activity comparable to TH-6038 (**2**).

Generally, the heterocyclic analogues were less effective in house flies than in fall armyworms, although compounds **6**, **12**, and **17** completely inhibited growth in flies at 1 ppm. Also effective were compounds **11** and **28** (100% inhibition at 10 ppm) and compounds **10**, **20**, and **25** (ca. 30–40% inhibition at 10 ppm). Although all of these analogues were superior to TH-6038 (0% inhibition at 10 ppm), none was as active as diflubenzuron which completely inhibited growth at 0.01 ppm. An interesting observation is the fact that four out of eight compounds effective against flies were pyridine analogues.

Since diflubenzuron is known to interfere with the reproductive performance of various insect species (Flint and Smith, 1977; Moore and Taft, 1975; Wright and Harris, 1976; Wright and Spates, 1976), we felt it worthwhile to evaluate the heterocyclic analogues for similar activity. Although feeding and injection methods were used to assess inhibition of reproduction in flies, the latter is felt to be more reliable since oral administration

often presents problems such as nonuniform uptake of the test compound by the flies.

The most effective reproduction inhibitors injected into female flies were the two pyridine analogues **11** and **12** (71 and 75% inhibition of hatch, respectively, at 10 μg/fly). Less effective but still possessing appreciable sterilizing activity were the thiazole analogue **5**, the pyrimidine **18**, and the benzoxazole **22**. None of the compounds were tested against males since diflubenzuron had no effect on males by the injection method.

In house fly feeding tests, only **11** and **25** inhibited reproduction: i.e., both completely inhibited pupal formation at dietary concentrations of 0.5 and 1%, respectively. Treated males were unaffected by either compound at similar dietary concentrations. Despite the sterilizing effectiveness of the aforementioned compounds in flies, none was as effective as diflubenzuron which completely inhibited hatch when administered by injection (10 μg/fly) or orally (0.1%).

None of the compounds, including diflubenzuron, induced sterility in fall armyworms by our test procedure. Perhaps sublethal concentrations of compounds in the larval medium were too low to induce sterility in the surviving adults.

In summary, we have identified some new heterocyclic analogues of diflubenzuron that markedly inhibit the growth of immature stages of the fall armyworm and house fly. Several of the compounds also inhibit reproduction of the house fly. Of those compounds tested, the 2-pyridyl derivatives were the most effective. From our results we speculate that further synthesis of analogues bearing the pyridine or thiazole systems could lead to compounds of even greater activity.

Supplementary Material Available: A listing of analytical data and recrystallization solvents for the heterocyclic analogues (2 pages). Ordering information is given on any current masthead page.

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Acute Toxicity and Developmental Effects of Analogues of Ethyl α-(4-Chlorophenoxy)-α-methylpropionate on Two Insects, *Oncopeltus fasciatus* and *Tenebrio molitor*

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Forty analogues of the hypocholesterolemic agent clofibrate were synthesized and bioassayed on the yellow mealworm, *Tenebrio molitor*, and the large milkweed bug, *Oncopeltus fasciatus*. The compounds demonstrated only weak morphogenic activity in *T. molitor* but moderate acute toxicity in *O. fasciatus*. The acute symptoms of clofibrate analogues in *O. fasciatus* were similar to those induced by the antijuvenile hormone precocene. Clofibrate analogues resulted in delayed or blocked development of *O. fasciatus* nymphs, yet failed to produce clear antijuvenile hormone activity.

Insect juvenile hormone (JH) mimics (juvenoids) hold promise as insect control agents which disrupt insect development or reproduction by inundating the insect's system with exogenous hormone or providing hormone activity at stages during development when the JH should be absent. The recent reports on anti-JH's (Bowers, 1976; Bowers et al., 1976) have stimulated interest that pest control agents may be developed which inhibit JH production. Such compounds may be found by attempting to inhibit key reactions in the JH biosynthetic pathway in vitro (Mumby et al., 1976), random screening with an appropriate in vivo bioassay (Bowers, 1976), or directed screening with an in vivo bioassay in an attempt to inhibit reactions in the JH biosynthetic pathway based on analogy with other organisms.

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