Larvicidal Effects of Benzoylphenylureas Against the Lesser Mealworm (Coleoptera: Tenebrionidae): Quantitative Structure-Activity Relationships[1,2]

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ABSTRACT Larvicidal activity of a congeneric set of *N*-2-halo- (and 2,6-dihalo) benzoyl-*N'*-phenylureas were determined in *in vivo* tests against the lesser mealworm, *Alphitobius diaperinus* (Panzer). Substituent modifications were made in both the benzoyl and anilide portions of the molecule. Linear regression analysis was used to derive quantitative structure-activity relationships (QSAR) from LC_{50} and LC_{99} values and a series of physicochemical substituent parameters. The analysis resulted in two significant single parameter regression equations selecting Hammett σ_p constant as the only relevant chemical descriptor and rejecting the other descriptors as insignificant. Penfluron and 2,6-difluoro-*N*-[[[4-(trifluoromethoxy)phenyl]amino]carbonyl]benzamide were potent larvicides in these tests and exceeded the effectiveness of diflubenzuron and triflumuron.

KEY WORDS Larvicides, lesser mealworm, benzoylphenylureas, chitin-synthesis inhibitors, *Alphitobius diaperinus*, QSAR, structure-activity relationships.

The lesser mealworm, *Alphitobius diaperinus* (Panzer), is a major pest to the poultry industry, particularly in broiler grow-out houses and high rise-caged layer houses (Pfeiffer and Axtell 1980). Although this insect has been implicated in the transmission of avian leukosis (Marek's disease) (Edison et al. 1966), the development of an effective vaccine for this costly disease in 1972 greatly lessened the economic impact of this insect to the poultry industry. Production practices in broiler grow-out houses induce high populations of the lesser mealworm, and this insect is now considered a major structural pest, particularly to polymer-based insulation panels (Turner 1986, Geden and Axtell 1987).

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Classical organophosphate, carbamate, pyrethroid (Vaughn and Turner 1984) and benzoylphenylurea-derived insecticides (Weaver and Kondo 1987, Miller and Redfern 1988) are effective against the lesser mealworm. Although previous benzoylphenylurea studies describe the potential usefulness of these growth regulators for control of the lesser mealworm (Retnakaran and Ennis 1985), little is known regarding structure-activity relationships (SAR) in this species. To identify these relationships, the larvicidal effectiveness of diflubenzuron and a series of congeners was determined, and biological activity indices (LC_{50}, LC_{99}) were correlated with substituent descriptors using stepwise regression analysis. Herein, we report the results of our studies.

Materials and Methods

Test Chemicals. Benzoylphenylureas were synthesized by one of two methods: (a) treatment of the appropriate 2-halo- (or 2,6-dihalo)benzoyl isocyanate with the corresponding substituted aniline or (b) treatment of the appropriate 2-halo- (or 2,6-dihalo)benzamide with the corresponding substituted phenyl isocyanate. Details describing these methods are published elsewhere (Wellinga et al. 1973a, 1973b). All compounds were recrystallized from acetonitrile or ethanol, prior to testing. Purity of test materials was estimated by TLC and melting point data and exceeded 98%. New compounds gave satisfactory (± 0.4% of theoretical) C, H, and N combustion analyses (Galbraith Laboratories, Knoxville, TN).

Larval Mortality Tests. Test insects were obtained from a laboratory colony of lesser mealworms that was established from insects collected from a commercial grow-out house on the eastern shore of Maryland. Conditions for colony maintenance were as follows: Mealworms were reared in polystyrene boxes (9.5 × 17 × 30 cm) containing sawdust and wood shaving litter. The insects were provided a diet of protein chicken ration. A relatively high humidity was maintained in the boxes by adding water to the litter. Test compounds were incorporated into a dietary medium comprised of the following: 80% *Tribolium* diet (white flour, whole-wheat flour and brewer's yeast, blended in a ratio of 4:4:1) and 20% chick starter. Prior to formulation, dietary components were passed through a 60-mesh sieve and then mixed together.

In initial tests, test compounds were formulated at a dietary concentration of 400 ppm. This was done by adding a small aliquot (≤ 100 μl) of an acetone-dimethyl sulfoxide (1:1 v/v) solution or suspension of the compound (finely pulverized) to the calculated amount of diet in a stainless-steel mixing bowl. At the same time, an acetone solution of the synergist piperonyl butoxide (PB) was added to the diet to provide a final concentration of 50 ppm of PB. Additional acetone was added to saturate the solid particles of diet, and the well-moistened mixture was thoroughly mixed. The acetone was then removed by evaporation in a fume hood (occasional stirring increased the evaporation rate).

Twenty-five grams of treated or untreated diet was placed into one-half pint mason jars. Moisture was provided by placing two 10-ml vials containing a 2% aqueous agar solution in the jars. Twenty-one-day-old lesser mealworm larvae were introduced into each jar (three replications); the jars were placed in a holding room maintained at 27° ± 1°C and 50 ± 5% RH. After approximately 30 days, the larvae were removed from the diet and counted. Percentage of larval mortality was

calculated by subtracting the number of living larvae in the treated jars from the number of living larvae in the untreated jars and dividing the number by the number of living larvae in the untreated jars.

To conduct regression analyses, it was necessary to determine LC_{50} and LC_{99} values from larval mortality data. LC_{50} values were determined as follows: depending on larval mortality observed at the initial test concentration (400 ppm), a range of concentrations was selected that allowed for determination of an approximate LC_{50} value. To refine this value, the compound was tested at several concentrations above and below the approximate LC_{50} value. Data were analyzed by probit analysis using SAS (1985) statistical software; LC_{99} values were also calculated by these methods.

Substituent Parameters for Regression Analysis. Values for substituent constants ΣE_s and $\Sigma\sigma_I$ used for benzoyl-ortho-substituents (Nakagawa et al. 1987), π, π^2, σ_p, F, R, MR, (Hansch and Leo 1979, Nakagawa et al. 1985), and ΔB_5 (Verloop et al. 1976, Nakagawa et al. 1984, Nakagawa et al. 1989) were obtained from the literature. Parameters are defined as follows: E_s, Tafts constant, characterizing steric effects of the substituent; σ_I, Charton's electronic constant, characterizing the electron-withdrawing power of the substituent; π, Hansch-Fujita hydrophobicity constant; σ_p Hammett constant characterizing the electron withdrawing power of the substituent in para position of the aromatic ring; F, R, Swain-Lupton electronic constants characterizing the field and resonance effects, respectively; B_5, STERIMOL steric parameter (Å) representing the maximum width from the axis connecting the carbon atom at the para position of the anilide benzene ring and the α atom of the substituent (in this analysis $\Delta B_5 = B_5(R) - B_5(H)$ values were used).

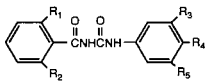
Results and Discussion

Larvicidal activity for 44 analogs of diflubenzuron, determined from an *in vivo* assay for *A. diapernus,* is shown in Tables 1 and 2. Quantitative structure-activity relationships were determined from larvicidal activity and selected electronic, steric, and hydrophobic substituent parameters through a stepwise regression analysis. To minimize variance in data caused by oxidative metabolism of the test compound (Nakagawa et al. 1984, 1989), the dietary medium was complemented with 50 ppm of the metabolic inhibitor PB. For the regression analysis, the dependent variables LC_{50} and LC_{99} [concentration of the test substance in ppm required to kill 50% or 99%, respectively, of the larvae] were transformed into A_{50} and A_{99}, by the following equations:

$$A_{50} = -\log(LC_{50}) + 3 \qquad A_{99} = -\log(LC_{99}) + 3 \qquad \text{(Table 1)}$$

To avoid chance correlation (Topliss and Costello 1972), a set of carefully selected chemical descriptors was used to derive regression equations. These descriptors have been used earlier to describe the QSARs for diflubenzuron analogs. The set included two descriptors (ΣE_s, $\Sigma\sigma_I$) for the benzoyl ortho-substituents R_1 and R_2 and seven descriptors (π, π^2, σ_p, F, R, MR, and ΔB_5) for the aromatic para-substituent position R_4 (Table 3). Only the compounds with reliable activity data (No.'s 1-18 and having a single para substituent on the anilide moiety (R_4) were included

Table 1. Larvicidal activity of benzoylphenylureas against the lesser mealworm.



Compd. No.	R_1	R_2	R_3	R_4	R_5	Larvicidal Activity (in ppm) LC_{50} obsd.	A_{50}* obsd.	A_{50}** calcd.	LC_{99} obsd.	A_{99}† obsd.	A_{99}‡ calcd.
1	F	F	H	Cl	H	3.88	2.41	1.96	10.89	1.96	1.62
2	F	F	H	CF_3	H	0.66	3.18	3.06	1.52	2.82	2.76
3	F	F	H	OCF_3	H	0.87	3.06	2.39	1.47	2.83	2.06
4	F	F	H	C_2H_5	H	151.93	0.82	0.62	327.18	0.48	0.22
5	F	F	H	i-C_3H_7	H	141.97	0.85	0.62	631.08	0.20	0.22
6	F	F	H	Br	H	4.37	2.36	1.96	39.44	1.40	1.62
7	F	F	H	H	H	346.07	0.46	1.15	700.25	0.15	0.77
8	F	F	H	OCH_3	H	255.04	0.59	0.20	772.97	0.11	-0.22
9	F	F	H	OC_6H_5	H	428.54	0.37	1.05	685.74	0.16	0.66
10	F	F	H	F	H	53.30	1.27	1.36	109.07	0.96	0.99
11	F	F	H	$C(CF_3)_2OH$	H	4.76	2.32	2.21	9.47	2.02	1.88
12	F	Cl	H	OCF_3	H	0.97	3.00	2.39	2.33	2.63	2.06
13	Cl	Cl	H	CF_3	H	2.49	2.60	3.06	4.72	2.33	2.76
14	Cl	Cl	H	C_2H_5	H	248.13	0.61	0.62	438.99	0.36	0.22
15	Cl	H	H	Cl	H	76.51	1.12	1.96	76.51	1.12	1.62
16	Cl	H	H	CF_3	H	1.25	2.90	3.06	1.96	2.71	2.76
17	Cl	H	H	OCF_3	H	3.23	2.49	2.39	6.01	2.22	2.06
18	Cl	H	H	$C(CF_3)_2OH$	H	13.97	1.85	2.21	30.59	1.51	1.88
19	F	F	CF_3	H	H	31.71	1.50	0	65.78	1.18	0
20	F	F	Cl	H	H	55.78	1.25	0	135.40	0.87	0
21	F	F	CF_3	Cl	H	4.75	2.32	0	11.63	1.93	0
22	F	F	CF_3	H	CF_3	2.84	2.55	0	5.48	2.26	0

* $A_{50} = \log (1/LC_{50}) + 3$.
** Calculated with Eq. 1.
† $A_{99} = \log (1/LC_{99}) + 3$.
‡ Calculated with Eq. 2.

Table 2. Larvicidal activity of benzoylphenylureas against the lesser mealworm.*

Compd. No.	R_1	R_2	R_3	R_4	R_5	LC_{50} (ppm)
23	F	F	H	n-C_4H_9	H	680**
24	F	F	H	t-C_4H_9	H	1053**
25	F	F	H	CO_2Et	H	1905**
26	F	F	OMe	H	H	3774**
27	F	F	Et	H	H	1905**
28	F	F	F	H	H	1802**
29	Cl	Cl	H	F	H	3571**
30	Cl	Cl	H	OMe	H	1198**
31	Cl	H	H	Et	H	1802**
32	Cl	H	H	H	H	1802**
33	F	F	H	OBz	H	†
34	F	F	H	N=NPh	H	†
35	F	F	CO_2Et	H	H	†
36	Cl	Cl	H	Cl	H	†
37	Cl	Cl	H	H	H	†
38	F	H	H	Cl	H	†
39	F	H	H	CF_3	H	†
40	Cl	H	H	OMe	H	†
41	Cl	H	H	N=NPh	H	†
42	Cl	H	H	F	H	†
43	H	H	H	H	H	†
44	H	H	H	Cl	H	†

* Structure shown in Table 1.
** Approximate values calculated from three data points.
† Inactive at 400 ppm.

in the regression analysis. Meta-substituted analogs (No.'s 19-22) were not represented in sufficient number to span the parameter descriptor space for the R_3 and R_5 substituent positions.

Regression analyses were carried out using the 1R and 2R (stepwise regression analysis) modules of the BMDP software (BMDP Statistical Software, Inc., 1988) to identify the possible significant regression equations for biological activity indices A_{50} and A_{99} of compounds No.'s 1-18 using nine chemical descriptors (ΣE_s, $\Sigma\sigma_I$, π, π^2,σ_p, F, R, MR, and ΔB_5 as independent variables (Table 3). The stepwise regression analyses resulted in two single parameter regression equations (Eq. 1 and Eq. 2), both cases selecting σ_p as the only relevant chemical descriptor and rejecting the other descriptors as insignificant.

Table 3. Substituent parameters for QSAR analysis.

Subst.	ΣE_s*	$\Sigma\sigma_I$*	$\pi^{\dagger}$	π^2	σ_p**	$F^{\dagger}$	$R^{\dagger}$	$\Delta B_5^{\dagger}$	$MR^{\dagger}$
H	-0.60	1.08	0.00	0.00	0.00	0.00	0.00	0.00	1.03
F	-0.60	1.08	0.19	0.04	0.06	0.43	-0.34	0.35	0.92
Cl	-0.60	1.08	0.88	0.78	0.23	0.41	0.44	0.80	6.03
Br	-0.60	1.08	1.02	1.04	0.23	0.44	-0.17	0.95	8.88
CF_3	-0.60	1.08	1.32	1.74	0.54	0.38	0.19	1.61	5.02
OCF_3	-0.60	1.08	1.04	1.08	0.35	0.38	0.00	2.61	7.86
Et	-0.60	1.08	0.82	0.67	-0.15	-0.05	-0.10	2.17	10.30
i-C_3H_7	-0.60	1.08	1.30	1.69	-0.15	-0.05	-0.10	2.17	14.96
OMe	-0.60	1.08	-0.26	0.07	-0.27	0.26	-0.51	2.07	7.87
OPh	-0.60	1.08	1.93	3.73	-0.03	0.34	-0.35	4.89	27.68
$C(CF_3)OH$			1.28	1.64	0.30	0.28	0.05	2.90	15.18

* Nakagawa et al. 1987.
** Hansch and Leo 1979, Nakagawa et al. 1985.
† Verloop et al. 1976, Nakagawa et al. 1984, Nakagawa et al. 1989.

$$A_{50} = 3.526\ (\pm 0.439)\ \sigma_p + 1.152 \quad (1)$$
$$n = 18 \qquad r = 0.895 \qquad s = 0.467 \qquad F = 64.5$$

$$A_{99} = 3.685\ (\pm 0.374)\ \sigma_p + 0.773 \quad (2)$$
$$n = 18 \qquad r = 0.926 \qquad s = 0.398 \qquad F = 96.9$$

In these equations, n represents the number of compounds, r is the correlation coefficient, s is the standard error of estimate, and F is the F-statistic for significance of the addition of each parameter term. Values in parentheses are for construction of the 95% confidence intervals.

In Equations 1 and 2, σ_p accounts for 80.12 and 85.83% of the variation in activity, respectively. None of the other chemical parameters had contributed significantly to the biological activity. The positive sign of the regression coefficient in both equations indicates that electron-withdrawing substituents enhance activity. Similar correlation for σ_p was reported in several earlier QSAR studies with *Pieris brassicae* L. (Verloop and Ferrell 1977) and *Chilo suppressalis* Walker (Nakagawa et al. 1984, 1985, 1991). While hydrophobicity and STERIMOL parameters played an important role in influencing larvicidal activity in *P. brassicae* and *C. suppressalis,* surprisingly, no significant correlation ($P > 0.05$) was found for these descriptors with the A_{50} and A_{99} values in *A. diaperinus.*

High activity of benzoylphenylureas possessing electron-withdrawing substituents in the anilide ring, may, in part, be attributed to the fact that these substituents lessen metabolic susceptibility of the aromatic ring (Nakagawa 1984). In apparent contradiction, studies (Nakagawa et al. 1991) measuring the inhibition of uptake of *N*-acetyl-[1-^{14}C] glucosamine into the integument by benzoylphenylureas containing electron-donating substituents in the anilide moiety, showed that the electron-donating effect intrinsically favors inhibition of chitin synthesis.

Compounds No.'s 23-32 (Table 2), most of which contained electron-donating substituents, elicited relatively low larvicidal activity. However, due to low activity, precise LC_{50} values for these analogs could not be calculated. Because alkyl or alkoxy substituents are also sensitive to metabolism, their presence in certain compounds may account for the observed low activity. Apparently, PB added to the diet in this study was not successful in fully suppressing metabolism of the alkyl- (or alkoxy) substituted benzoylphenylureas in *A. diapernus.*

Compounds No.'s 33-44 (Table 2) were inactive at the highest concentration tested. Inactivity for some of these analogs was entirely unexpected. These anomalies may result from (a) higher susceptibility of these compounds to metabolic oxidation, (b) secondary modes of action, or (c) experimental errors such as non-homogeneity of particle size of the compound in the diet (Verloop and Ferrell 1977).

In summary, a stepwise regression analysis was used for the first time to identify the relevant substituent chemical descriptors correlating with the larvicidal activity of a congeneric set of benzoylphenylureas for the lesser mealworm. Specifically, we found that strong electron-withdrawing substituents, in the para position of the aniline ring, exerted the greatest influence on larvicidal activity. This correlation was consistent with previous QSAR studies in unrelated insect species. Data from these studies also show that eight analogs were more effective larvicides than diflubenzuron (No. 1).

Moreover, consistent with previous studies (Miller and Redfern 1988), this study showed that penfluron (No. 2) and the trifluoromethoxy analog (No. 3), 2,6-difluoro-*N*-[[[4-(trifluoromethoxy)phenyl]amino]carbonyl]benzamide, are outstanding larvicides and offer considerable promise for control of the lesser mealworm.

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