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Quantitative Structure–Activity Analysis of Larvicidal 1-(Substituted benzoyl)-2-benzoyl-1-*tert*-butylhydrazines against *Chilo suppressalis**

Nobuhiro Oikawa, Yoshiaki Nakagawa,‡ Keiichiro Nishimura, Tamio Ueno & Toshio Fujita

Department of Agricultural Chemistry, Kyoto University, Kyoto 606-01, Japan

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Abstract: The larvicidal activity of a number of 1-(substituted benzoyl)-2-benzoyl-1-*tert*-butylhydrazines against the rice stem borer (*Chilo suppressalis* Walk.) was measured. Variations in the activity were examined quantitatively using physicochemical substituent and molecular parameters and regression analysis. The results indicated that the molecular hydrophobicity and the electron-withdrawing inductive/field effect of *ortho* substituents are favourable to larvicidal activity. The bulkiness of substituents at the *meta* and *para* positions was unfavourable to activity, substitution at the *para* position being more unfavourable than that at the *meta* position in terms of van der Waals' volume. The 2,3-, 2,5- and 2,6-disubstitution patterns were also unfavourable to activity. Reductions in larvicidal activity caused by the 2,6-, 2,3,5- and 2,3,4,5-substitutions were greater than those induced by the 2,3- and 2,5-disubstitutions. When the sum of contributions from favourable effects is greater than that from unfavourable effects, the larvicidal activity is expected to be superior to that of the unsubstituted compound.

1 INTRODUCTION

Key steps in insect metamorphosis and development are regulated by juvenile and moulting hormones. New insecticides that regulate the action of these hormones are specific to insects, and scarcely toxic to mammals. A number of juvenile hormone mimics have been synthesized[1–7] and some of them are commercially available as larvicides. In contrast, progress towards finding a new class of insecticides related to moulting hormones has been hampered by the structural complexity.[8–10]

Recently, RH-5849 (Fig. 1; $X_n = H$) was found to mimic the action of a moulting hormone, 20-hydroxyecdysone, in *Drosophila* Kc cells at the receptor level.[11,12] The external administration of an excessive amount of the moulting hormone or its mimics has been shown to induce severe damage to larval growth, leading to death.[13–15] In oral administration tests with tobacco hornworms, RH-5849 was over 670 times more potent as the moulting hormone than 20-hydroxyecdysone.[16] Although various substituents have been introduced into benzene rings of the compound to optimize moulting-hormone as well as insecticidal activity,[17] the effects of substituents on activity are little understood.

In this study, we synthesized a number of substituted dibenzoyl hydrazines in which X_n is varied (Fig. 1) and measured their larvicidal activity against the rice stem borer (*Chilo suppressalis* Walk.). With a traditional procedure to examine the structure–activity relationship quantitatively[18] that uses physicochemical substituent and molecular parameters and regression analysis, we found that hydrophobic, steric and electronic effects of

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‡ To whom correspondence should be addressed.

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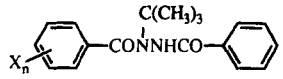


Fig. 1. Structure of the larvicidal 1-(substituted benzoyl)-2-benzoyl-1-*tert*-butylhydrazines studied.

substituents as well as factors probably related to the molecular conformation are decisive of variations in the activity.

2 EXPERIMENTAL

2.1 Synthesis

The final compounds and intermediates were prepared following the typical procedures shown in Fig. 2. Some representative synthetic procedures are described below. Yields were not optimized. Most of the benzoic acids used to derive final dibenzoylhydrazines are commercially available. The dibenzoylhydrazines are listed in Table 1 with their uncorrected melting point. The chemical structure of each compound was confirmed by proton nuclear magnetic resonance ([^{1}H]NMR) spectra and elemental analyses. The [^{1}H]NMR spectra were recorded on a JEOL JNM-PMX60 spectrometer in deuterochloroform ($CDCl_3$) and/or deuterodimethyl sulfoxide (DMSO-d_6) with tetramethylsilane as the internal standard. Each of the analytical values for C, H, and N agreed within 0·3% with the calculated value.

2.1.1 Procedure A:[19] *preparation of 2-methyl-3-chlorobenzonitrile*

Sodium nitrite (1·2 g) dissolved in water (4 ml) was added to a mixture of 2-methyl-3-chloroaniline (2·82 g), 35% hydrochloric acid (7·0 ml), and ice (20 g). The reaction mixture was stirred vigorously to prepare the corresponding diazonium solution, which was then added dropwise to a solution prepared from copper(I) cyanide (5·5 g), potassium cyanide (13 g) and water (30 ml) under ice-cold conditions. The reaction mixture was stirred for 20 min at room temperature followed by heating, and then subjected to steam distillation. The distillate was extracted with diethyl ether and the solvent was evaporated to afford the benzonitrile, which was recrystallized from ethanol + water. Yield was 1·15 g (37·9%). [^{1}H]NMR ($CDCl_3$) δ: 2·52 (3H, s), 7·10–7·60 (3H, m) ppm.

2.1.2 Procedure B: preparation of 2-methyl-3-chlorobenzoic acid

2-Methyl-3-chlorobenzonitrile (1·35 g) was stirred overnight at 70°C in 70% sulfuric acid (7·0 ml). After addition of water (50 ml) to the reaction mixture, the precipitated amide crystals were collected by filtration, washed with water, and dried. Sodium peroxide (1·56 g) was added slowly to the amide (1·2 g) suspended in water (30 ml) and stirred for 1 h at room temperature. The reaction mixture was neutralized with hydrochloric acid and the precipitates collected by filtration. The crystals were repeatedly washed with water and dried to afford the corresponding benzoic acid. Yield was 0·36 g (23·7%). [^{1}H]NMR (DMSO-d_6) δ: 2·46 (3H, s), 7·26–7·40 (3H, m), 10·20–10·90 (1H, br) ppm. The corresponding benzoic

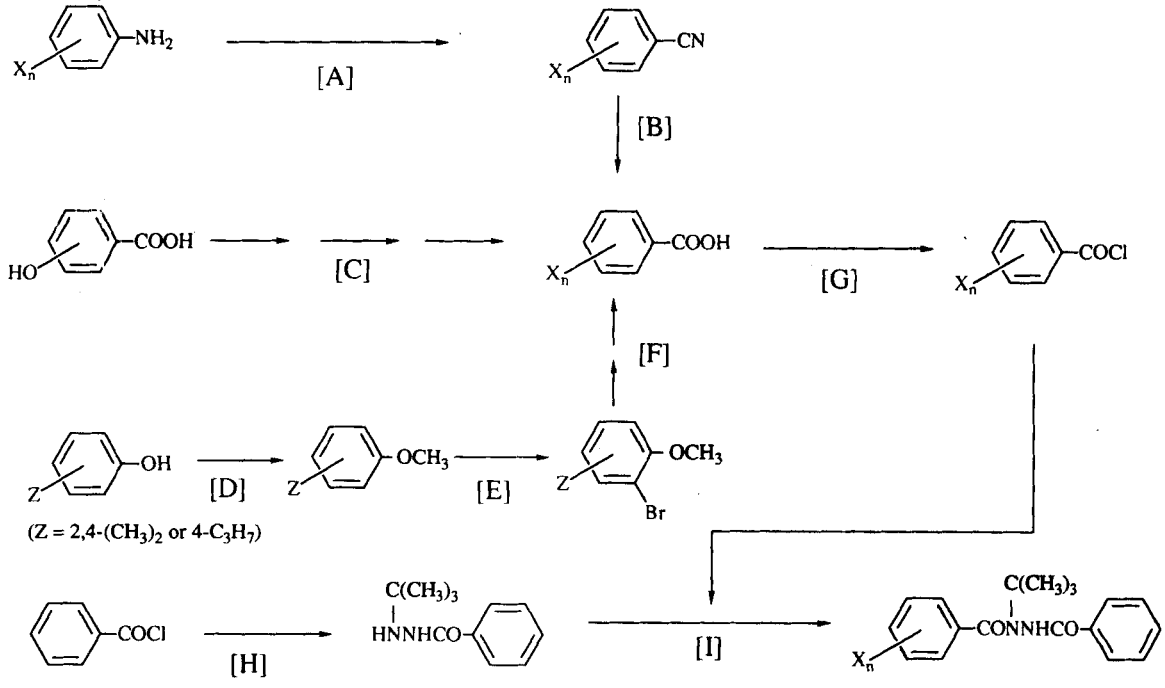


Fig. 2. Synthetic scheme for 1-(substituted benzoyl)-2-benzoyl-1-*tert*-butylhydrazines.

acids for compounds **36**, **38**, **52** and **53** were synthesized according to procedures A and B.

2.1.3 Procedure C:[20] *preparation of 2-benzyloxybenzoic acid*

Thionyl chloride (20 ml) was added to salicylic acid (25·0 g) suspended in ethanol (100 ml) and the mixture was stirred overnight. After removing ethanol and excess thionyl chloride under reduced pressure, the solid material was dissolved in diethyl ether and washed with saturated sodium carbonate and brine. After drying the ethereal portion over anhydrous sodium sulfate, the solvent was evaporated to afford ethyl salicylate (29·1 g). Benzyl bromide (5·5 g) and solid potassium hydroxide (2·0 g) was added to ethyl salicylate (5·0 g) dissolved in dimethyl sulfoxide (50 ml). After the mixture had been stirred for 5 h at 45°C, it was diluted with water (200 ml), extrated with diethyl ether and washed with saturated sodium carbonate and brine. After drying the ethereal solution with sodium sulfate, the solvent was evaporated to afford ethyl 2-benzyloxybenzoate, 7·0 g (89·7%). This benzoate was dissolved in aqueous sodium hydroxide (2 M; 120 ml) and tetrahydrofuran (50 ml) and refluxed overnight. The reaction mixture was neutralized with hydrochloric acid and the solvent was evaporated. The residue was filtered and washed repreatedly with water to afford the corresponding acid. Yield was 5·36 g (81·3%). [^{1}H]NMR ($CDCl_3$) δ: 5·73 (2H, s), 7·40–8·80 (9H, m), 9·40–9·90 (1H, br) ppm. With similar procedures, 2-methylthiobenzoic and 4-phenylpropyloxybenzoic acids as intermediates of compounds **14** and **35**, respectively, were prepared.

2.1.4 Procedure D: preparation of 2,4-dimethylanisole

Sodium hydride (8·5 g as 60% suspension in oil), was slowly added to 2,4-dimethylphenol (25·0 g) dissolved in *N*,*N*-dimethylformamide (50 ml) and stirred for 10 min. Methyl iodide (30·0 g) was added dropwise to this mixture and stirred for 5 h at room temperature. The reaction mixture was diluted with water (200 ml) and extracted with diethyl ether. The ether layer was washed with saturated sodium carbonate solution and brine and dried over anhydrous sodium sulfate. The solvent was evaporated to afford a colourless oil. Yield was 29·8 g (quantitative). [^{1}H]NMR ($CDCl_3$) δ: 2·33 (6H, s), 3·90 (3H, s), 7·20–7·50 (3H, br) ppm.

2.1.5 Procedure E: preparation of 2,4-dimethyl-6-bromoanisole

Bromine (4·8 g) was added dropwise within 1 h to 2,4-dimethylanisole (4·0 g) dissolved in acetic acid (30 ml) and stirred for 5 h at 50°C in the presence of a catalytic amount of iron powder. The reaction mixture was extracted with diethyl ether and the ether layer was washed with 8% sodium thiosulfate, saturated sodium carbonate solution, and brine in that order. The ethereal portion was dried over anhydrous sodium sulfate and the solvent was evaporated to afford an oil. The oil was purified by a silica gel column chromatography (Wakogel C-200, eluted with hexane + ethyl acetate, 9 + 1 by volume) to afford 2,4-dimethyl-6-bromoanisole as a colourless oil. Yield was 1·62 g (25·6%). [^{1}H]NMR ($CDCl_3$) δ: 2·33 (6H, s), 3·90 (3H, s), 7·20 (1H, s), 7·50 (1H, s) ppm.

2.1.6 Procedure F:[21] *preparation of 2-methoxy-3,5-dimethylbenzoic acid*

2,4-Dimethyl-6-bromoanisole (1·62 g) dissolved in anhydrous ether (10 ml) in a 50-ml flask sealed with a rubber septum cap was cooled by dry ice in acetone. *n*-Butyl lithium (10 ml) was added with use of a syringe through the rubber cap and stirred for 30 min. An excess of dry ice was added to the reaction mixture and stirred. The reaction mixture was diluted with hydrochloric acid (1 M; 30 ml) and extracted with ethyl acetate. The ethyl acetate portion was dried over anhydrous sodium sulfate after washing with brine and the solvent evaporated. The resultant residue was recrystallized from hexane to afford 2-methoxy-3,5-dimethylbenzoic acid. Yield was 0·52 g (36·8%). [^{1}H]NMR ($CDCl_3$) δ: 2·33 (6H, br), 3·90 (3H, br), 7·20 (1H, br), 7·55 (1H, br), 10·4–10·9 (1H, br) ppm. 2-Methoxy-5-*n*-propylbenzoic acid for compound **42** was synthesized according to procedures D, E and F.

2.1.7 Procedure G: preparation of 2-methoxybenzoyl chloride

The mixture of 2-methoxybenzoic acid (5·0 g) and thionyl chloride (7·0 ml) was refluxed for 12 h and the excess thionyl chloride removed under reduced pressure. The residue was distilled under vacuum to afford a yellowish oil. Yield was 4·82 g (86·2%). Each of the substituted benzoic acids was chloridated by this procedure.

2.1.8 Procedure H:[17] *preparation of* N-*benzoyl*-N′-tert-*butylhydrazine*

Benzoyl chloride (22·6 g) and aqueous sodium hydroxide (6 M; 60 ml) were simultaneously added to *tert*-butylhydrazine hydrochloride (20·0 g) dissolved in diethyl ether (250 ml) over 30 min and stirred for 1 h at room temperature. The ether layer of the reaction mixture was washed with aqueous sodium hydroxide (1 M) and brine, and the basic monobenzoylated hydrazine was extracted with aqueous hydrochloric acid. The extract was washed with hexane and neutralized with sodium hydroxide (6 M) to afford crystals, which were recrystallized from hexane and diethyl ether. Yield was 26·2 g (84·4%). [^{1}H]NMR ($CDCl_3$) δ: 1·20 (9H, s), 1·49 (1H, s), 5·33–6·20 (1H, br), 7·10–8·00 (5H, m) ppm.

2.1.9 Procedure I:[17] *preparation of* N-*benzoyl*-N′-(*4-chlorobenzoyl*)-N′-tert-*butylhydrazine*

4-Chlorobenzoyl chloride (0·42 g) and aqueous sodium hydroxide (1 M; 5 ml) were simultaneously added to

N-benzoyl-*N'*-*tert*-butylhydrazine (0·29 g) dissolved in diethyl ether (20 ml) in 30 min and stirred for 3 h at room temperature. The reaction mixture was extracted with diethyl ether, and the ether portion was washed with sodium hydroxide (1 M), hydrochloric acid (1 M), and brine in that order. The ethereal extract was dried over anhydrous sodium sulfate and the solvent was evaporated to give a residue which was recrystallized from hexane and diethyl ether to afford the corresponding dibenzoylhydrazine. Yield was 0·12 g (24%). [^{1}H]NMR ($CDCl_3$ and DMSO-d_6) δ: 1·52 (9H, s), 7·00–7·60 (9H, m), 9·80 (1H, s) ppm.

2.2 Larvicidal activity

The method for the larvicidal test against the rice stem borer was similar to that described previously.[22,23] Third-instar larvae about 10 days after hatching were reared on an agar diet containing 1×10^{-4} M reagent grade piperonyl butoxide (PB) purchased from Tokyo Kasei Co., unless otherwise noted, for at least 1 h. PB is an inhibitor of oxidative metabolism and its addition in the diet has been shown to elevate the larvicidal activity level of insect growth regulators such as substituted benzoylphenylureas under conditions similar to those used in this study against the rice stem borer.[23] Various doses (usually four) of each compound in 0·5 μl of dimethyl sulfoxide were applied topically to the dorsal part of 20 larvae, which were further reared on the same diet at 28°C under long-day photoperiods (16:8 h, light:dark). The larvicidal activity in terms of pLD_{50}, the log value of the reciprocal of the doses (mmol per insect) required to kill 50% of the larvae 7 days after the application, was estimated by probit transformation.[24,25] The pLD_{50} value was determined for all of the compounds for which it could be measured. The number of repetitions (n) and the pLD_{50} values are shown in Table 1.

2.3 Physicochemical substituent and molecular parameters

Physicochemical parameters of substituents on the benzoyl-benzene ring are listed in Table 2. For *ortho* substituents, the Charton σ_I[26] which represents the inductive/field component of their electronic effect was used. As the steric parameter, V_w, expressing the van der Waals' volume which is calculated according to Bondi,[27] was used. In the analyses ΔV_w [$= V_w(X) - V_w(H)$] was used for simplicity, where $V_w(X)$ and $V_w(H)$ are those of X-substituent and H, respectively. The ΔV_w value used here is scaled by 0·1 to make the size comparable to that of other parameters. For the steric effect of substituents, we have examined other parameters such as STERIMOL parameters,[28] E_s defined by Taft, Kutter and Hansch,[29] and MR (substituent molar refractivity).[30] The best result was derived with the Bondi volume V_w. Most ΔV_w values are cited from one of our previous publications.[31]

The molecular hydrophobicity parameter, $\Delta \log P$, was defined as the difference in $\log P$ between compounds with substituent(s) X_n and that of the unsubstituted compound **1** as:

$$\Delta \log P = \log P[\mathrm{Ph}(X_n)\mathrm{CON}(\textit{tert}\text{-Bu})\mathrm{NHCOPh}] - \log P \text{ (compound 1)} \quad (1)$$

The $\log P$ value (P is the partition coefficient in *n*-octanol/water system) for most of the compounds was experimentally measured according to the reported procedure.[32] Log P values for compounds with a side chain such as the hydrazine bridge capable of hydrogen bonding are susceptible to stereo-electronic effects of substituents on the benzene ring.[32–34] Taking this into account, the experimentally measured $\Delta \log P$ values for 45 compounds were analyzed to give eqn (2).

$$\Delta \log P = 0{\cdot}858(\pm 0{\cdot}095)\Sigma\pi(X_i) + 0{\cdot}690(\pm 0{\cdot}161)\Sigma\sigma^0 - 0{\cdot}726(\pm 0{\cdot}213)\Sigma\sigma_I^{ortho} + 0{\cdot}259(\pm 0{\cdot}104)\Sigma E_s^{ortho} + 0{\cdot}079(\pm 0{\cdot}087) \quad (2)$$

$$n = 45 \quad s = 0{\cdot}159 \quad r = 0{\cdot}958 \quad F_{4,40} = 111{\cdot}91$$

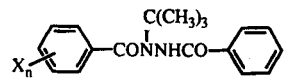
In this and the following equations, n is the number of compounds, s is the standard deviation, r is the correlation coefficient, and F is the value of the ratio between regression and residual variances. The figures in parentheses following each coefficient are the 95% confidence intervals of the regression coefficient. In eqn (2), $\Sigma\pi(X_i)$ is the sum of the reference hydrophobicity parameter for substituent(s) X_i evaluated from $\log P$ values of mono-substituted benzenes.[35] The σ^0 cited from our previous publications[33,34] is the parameter representing the electron-withdrawing property of substituents devoid of the through resonance effect. E_s is a set of steric parameters including those defined by Taft for alkyl substituents and those extended by Kutter and Hansch to heteroatomic substituents.[29] The reference point of E_s value was shifted to that of H. $\Sigma\sigma^0$ is the summation of the σ^0 values at *ortho*, *meta*, and *para* positions. The $\sigma^0(ortho)$ is taken as equivalent with $\sigma^0(para)$.[33] $\Sigma\sigma_I^{ortho}$ and ΣE_s^{ortho} are the summations of respective values only for *ortho* substituents.[33] Substituent parameters to derive eqn (2) are listed in Table 2. The $\log P$ values calculated by eqn (2) and the experimentally measured values are listed in Table 1. For compounds without the experimentally measured $\log P$, that calculated by eqn (2) was used in the correlation analyses.

3 RESULTS

3.1 Larvicidal activity against rice stem borers

As a preliminary, we examined the effect of the synergist, PB, for dibenzoylhydrazines. The activities for the

TABLE 1
Larvicidal Activity of Dibenzoylhydrazines against the Rice Stem Borer and Hydrophobicity of Compounds



Compounds			*Larvicidal activity* pLD_{50} *(mmol insect*$^{-1}$*)*		
No.	X_n	*mp* (°C)	*Observed*[a]	*Calculated*[b]	log *P*
1	H	178–179	6·27 (±0·16) (*n* = 5)	5·95	2·45
2	2-F	183–184	6·24 (±0·16) (*n* = 2)	6·70	2·38
3	2-Cl	161–162	6·83 (±0·36) (*n* = 5)	6·78	2·59
4	2-Br	195–196	6·88 (±0·23) (*n* = 3)	6·86	2·69
5	2-I	188–189	6·99 (±0·11) (*n* = 2)	6·88	2·83
6	2-CF_3	172–173	6·90 (±0·23) (*n* = 4)	6·90	2·85
7	2-NO_2	181–182	6·80 (±0·02) (*n* = 2)	6·80	2·27
8	2-CH_3	214–215	5·82 (±0·16) (*n* = 2)	6·20	2·75
9	2-C_2H_5	177–178	6·03 (*n* = 1)	6·38	2·96
10	2-C_6H_5	144–145	<5·00 (0%) (*n* = 1)	7·39	3·89[c]
11	2-OCH_3	181–182	6·14 (±0·06) (*n* = 2)	6·04	2·04
12	2-$OCH(CH_3)CH_2CH_3$	116–117	<4·00 (0%) (*n* = 1)	7·01	3·18[c]
13	2-$OCH_2C_6H_5$	89–90	<4·00 (0%) (*n* = 1)	7·66	3·66[c]
14	2-SCH_3	196–198	<5·00 (45%) (*n* = 1)	6·53	2·60
15	3-F	176–177	6·31 (±0·12) (*n* = 2)	6·13	2·78
16	3-Cl	180–181	6·45 (±0·06) (*n* = 3)	6·37	3·28
17	3-Br	217–218	6·49 (±0·15) (*n* = 2)	6·45	3·49
18	3-I	255–256	6·58 (*n* = 1)	6·51	3·72
19	3-CF_3	203–204	6·02 (±0·02) (*n* = 2)	6·34	3·61
20	3-NO_2	222–223	5·38 (±0·03) (*n* = 2)	5·73	2·73
21	3-CN	173–174	4·68 (±0·06) (*n* = 2)	5·45	2·34
22	3-CH_3	210–212	6·10 (±0·30) (*n* = 4)	5·88	2·79
23	3-OCH_3	188–189	5·89 (±0·23) (*n* = 2)	5·58	2·56
24	4-F	217–218	6·44 (*n* = 1)	6·03	2·85
25	4-Cl	212–213	6·55 (±0·11) (*n* = 2)	6·02	3·42
26	4-Br	228–229	5·94 (*n* = 1)	5·98	3·66
27	4-I	234–235	5·51 (±0·16) (*n* = 2)	5·72	3·78
28	4-CF_3	228–229	5·58 (*n* = 1)	5·53	3·77
29	4-NO_2	250–251	<5·00 (0%) (*n* = 1)	4·94	2·63
30	4-CN	199–200	5·09 (*n* = 1)	5·00	2·50
31	4-CH_3	187–188	5·61 (±0·02) (*n* = 2)	5·51	2·99
32	4-$C(CH_3)_3$	200–201	<4·48 (0%) (*n* = 1)	4·00	4·11[c]
33	4-C_6H_5	215–216	<4·48 (0%) (*n* = 1)	3·99	4·24[c]
34	4-OCH_3	221–222	4·76 (*n* = 1)	4·87	2·56
35	4-$O(CH_2)_3C_6H_5$	161–162	<4·48 (0%) (*n* = 1)	1·45	4·50[c]
36	2,3-Cl_2	209–210	6·33 (±0·04) (*n* = 2)	6·25	3·41
37	2-CH_3, 3-Cl	213–214	5·76 (*n* = 1)	5·67	3·57
38	2,3-$(CH_3)_2$	204–205	4·54 (±0·01) (*n* = 2)	5·20	3·10
39	2,4-Cl_2	134–135	7·01 (±0·05) (*n* = 2)	6·85	3·55
40	2,4-$(CH_3)_2$	195–196	4·92 (*n* = 1)	5·66	3·18
41	2,5-Cl_2	213–214	6·36 (±0·03) (*n* = 2)	6·21	3·36
42	2-OCH_3,5-C_3H_7	122–123	5·21 (±0·11) (*n* = 2)	5·20	3·32[c]
43	2,5-$(CH_3)_2$	197–198	5·64 (±0·09) (*n* = 2)	5·34	3·25
44	2,6-F_2	194–195	5·02 (±0·17) (*n* = 2)	4·82	2·16
45	2-F,6-Cl	209–210	5·10 (*n* = 1)	4·87	2·34
46	2,6-Cl_2	198–199	4·52 (*n* = 1)	4·96	2·56
47	3,4-Cl_2	238–239	6·78 (±0·25) (*n* = 4)	6·45	4·25
48	3,4-$(CH_3)_2$	225–226	5·52 (±0·02) (*n* = 2)	5·45	3·34
49	3,4-$(OCH_3)_2$	205–206	<5·00 (0%) (*n* = 1)	3·99	2·09
50	3,5-Cl_2	259–260	7·07 (±0·09) (*n* = 3)	6·92	4·26[c]
51	3,5-$(CH_3)_2$	206–207	6·43 (±0·19) (*n* = 3)	6·04	3·39
52	2,3,4-Cl_3	209–210	6·17 (±0·16) (*n* = 2)	6·34	4·39[c]
53	2,5-Cl_2,3-CF_3	185–186	5·28 (*n* = 1)	5·80	4·68[c]
54	2-OCH_3,3,5-$(CH_3)_2$	92–93	4·72 (*n* = 1)	4·20	2·91[c]
55	2,3,4,5-F_4	158–159	5·38 (±0·03) (*n* = 2)	5·27	3·44
56	2,3,4,5,6-F_5	200–201	<4·48 (0%) (*n* = 1)	3·42	3·25

[a] With the mean standard deviation for the number of repeated runs indicated by *n*. Percentages in parentheses are kills at the stated dose.
[b] Calculated by eqn (4).
[c] Calculated by eqn (2).

TABLE 2
Physicochemical Parameters for Substituents

Substituent	π^a	σ^0_{meta}	σ^0_{para}	σ_I	E_s	ΔV_w
H	0·00	0·00	0·00	0·00	0·00	0·00
F	0·14	0·35	0·17	0·54	−0·46	0·33
Cl	0·71	0·37	0·27	0·47	−0·97	0·95
Br	0·86	0·38	0·26	0·47	−1·16	1·26
I	1·12	0·35	0·27	0·40	−1·40	1·71
CF_3	0·88	0·47	0·53	0·40	−2·40	1·94
NO_2	−0·28	0·70	0·82	0·67	−1·01	1·43
CN	−0·57	0·62	0·69	0·57	−0·51	1·22
CH_3	0·56	−0·07	−0·12	−0·01	−1·24	1·12
C_2H_5	1·02	−0·07	−0·13	−0·01	−1·31	2·14
C_3H_7	1·55	−0·07	−0·13	−0·01	−1·60	3·16
$C(CH_3)_3$	1·98	−0·07	−0·17	−0·01	−2·78	4·18
C_6H_5	1·96	0·10	0·04	0·12	−1·01	4·33
OCH_3	−0·02	0·06	−0·16	0·30	−0·55	1·44
$OCH(CH_3)CH_2CH_3$	1·30[b]		−0·17[c]	0·28	−0·55	4·50
$OCH_2C_6H_5$	1·66		0·23[d]	0·43	−0·55	5·68
$O(CH_2)_3C_6H_5$	2·41[b]		−0·14[e]	0·28[e]	−0·55	7·72
SCH_3	0·61	0·13	0·06	0·30	−1·07	2·20

[a] From Refs 32 and 35, unless otherwise noted.
[b] Calculated by CLOGP program (Ref. 35).
[c] The value for $OCH(CH_3)_2$ was used.
[d] Hammett σ value was used.
[e] The value of OC_2H_5 was used.

unsubstituted compound **1** and compounds with electron-withdrawing (compound **3**) and -donating substituents (compound **11**) measured without the synergist were 6·16, 6·55 and 5·82, respectively. The activities measured in the presence of PB (Table 1) of these compounds were higher than the unsynergistic activity by 0·1–0·2 log units. Thus, the activity of other compounds was measured under synergistic conditions with PB to eliminate perturbations in the activity index as far as possible.

Among mono-substituted derivatives (compounds **1–35**), the effect of electron-withdrawing substituents such as halogens, CF_3, and NO_2 at the *ortho* position was most prominent in increasing activity. The effect of electron-donating alkyl and alkoxy groups was not significant. At the *meta* position, halogens were favourable to activity, whereas bulkier electron-withdrawing groups such as CF_3, NO_2 and CN were unfavourable. Introduction of F and Cl at the *para* position slightly increased activity, but bulkier halogens and electron-withdrawing groups such as CF_3, NO_2 and CN decreased activity. Substitution by such groups as alkyl and alkoxy at *meta* and *para* positions somewhat reduced activity. The bulkier substituents (in compounds **10**, **12–14**, **32**, **33** and **35**) were incompatible with activity.

The activity of multisubstituted compounds (compounds **36–56**) varied drastically, depending upon types and patterns of substitution. The substituent effects were not generalized except for the following aspects. Among the disubstituted compounds **36–51**, 2,4- and 3,5-dichloro derivatives (compounds **39** and **50**) had higher levels of activity. Compounds with two methyl groups (compounds **38**, **40**, **43**, **48** and **51**) were not so potent as the corresponding dichloro derivatives (compounds **36**, **39**, **41**, **47** and **50**). 2,6-Disubstituted compounds **44–46** and tri-, tetra- and pentasubstituted compounds **52–56** were less potent than unsubstituted compound **1** (RH-5849). The activity of compounds **49** and **56** was too low to be measured.

3.2 Quantitative analysis with substituent parameters

The effects of substituents on the activity were not simple as indicated above. Various effects seemed to overlap, showing a very complicated structure–activity pattern. First, we analysed the activity of mono-substituted compounds. The preliminary analyses for *ortho*, *meta* and *para* derivatives separately indicated that the molecular hydrophobicity and the position-specific electronic and steric effects of substituents are significant at various levels. For the combined set of monosubstituted compounds, eqn (3) was formulated as giving the best correlation quality.

$$\begin{aligned} pLD_{50} = {} & 0{\cdot}977(\pm 0{\cdot}314)\,\Delta \log P + 1{\cdot}280(\pm 0{\cdot}739)\sigma_I^{ortho} \\ & - 0{\cdot}480(\pm 0{\cdot}265)\,\Delta V_w^{meta} \\ & - 0{\cdot}890(\pm 0{\cdot}289)\,\Delta V_w^{para} \\ & + 6{\cdot}010(\pm 0{\cdot}275) \qquad (3) \end{aligned}$$

$$n = 27 \quad s = 0{\cdot}300 \quad r = 0{\cdot}899 \quad F_{4,22} = 23{\cdot}29$$

In this and the following equations, σ_I^{ortho} is for the inductive/field effect of *ortho* substituents as in eqn (2), while the superscripts *meta* and *para* associated with the ΔV_w terms indicate that the terms are for *meta* and *para* derivatives, respectively. In deriving this and the following equations, compounds whose activity was too low to measure (compounds **10**, **12–14**, **29**, **32**, **33** and **35**) were not included. Addition of the electronic effect terms for *meta* and *para* substituents as well as the steric effect for *ortho* substituents did not improve eqn (3). The use of other steric parameters such as Verloop's STERIMOL parameters and Taft–Kutter–Hansch E_s in the place of and in addition to ΔV_w did not improve the correlation as far as the compounds for which the activity was measurable were concerned.

Comparison of the observed activity values of multi-substituted compounds with the activity values estimated by eqn (3), assuming that the effects of substituents were additive, showed that the activity of 2,4-, 3,4- and 3,5-disubstituted derivatives (compounds **39**, **40** and **47–51**) was approximately in accord to that estimated. But the activities of 2,3- and 2,5-disubstituted compounds **36–38**, **41–43** as well as 2,3,4-trisubstituted compound **52** were lower and those of compounds **53–55** with 2,3,5-trisubstituted patterns and 2,6-disubstituted compounds **44–46** were even lower than the corresponding values calculated by eqn (3) (Fig. 3). By considering these results and including all mono- and multisubstituted compounds

TABLE 3
Development of Equation (4)

Intercept	$I_{2,6}$	$I_{2,3,5}$	$\Sigma\sigma_I^{ortho}$	$\Delta \log P$	ΔV_w^{para}	$\Sigma\Delta V_w^{meta}$	*s*	*r*	$F_{X,Y}$[a]
5·986	−1·106						0·688	0·376	$F_{1,44} = 7{\cdot}25$
6·127	−1·247	−0·468					0·639	0·527	$F_{1,43} = 8{\cdot}09$
5·940	−2·791	−0·727	1·715				0·540	0·704	$F_{1,42} = 18{\cdot}10$
5·619	−2·730	−0·940	2·017	0·474			0·479	0·782	$F_{1,41} = 12{\cdot}32$
5·748	−2·694	−1·103	1·872	0·675	−0·562		0·383	0·871	$F_{1,40} = 24{\cdot}33$
5·946	−2·501	−0·935	1·504	0·879	−0·325	−0·815	0·337	0·904	$F_{1,39} = 12{\cdot}48$

[a] F statistic for the significance of the addition of each parameter. X: The number of independent variables added at each step of the development, Y: *n*-*m*-1, *n* being the number of datum points and *m* being the total number of independent variables in the developed equation. $F_{1,60,0\cdot05} = 4{\cdot}00$, $F_{1,30,0\cdot05} = 4{\cdot}17$.

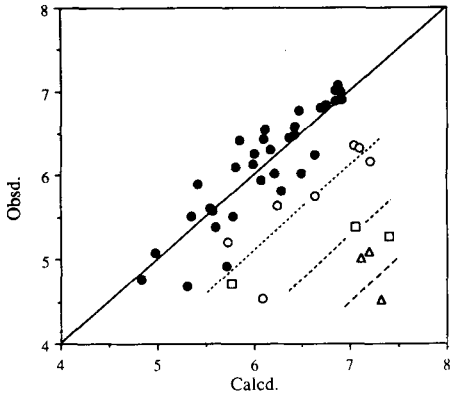


Fig. 3. Relationship between observed and calculated larvicidal activities of tested compounds against the rice stem borer. Calculated values were obtained from eqn (3). (●) Mono-substituted and 2,4-, 3,4- and 3,5-disubstituted compounds; (○) compounds having 2,3- or 2,5-substitution pattern; (□) compounds having both 2,3- and 2,5-substitution patterns; (△) compounds having 2.6-substitution pattern.

with measurable activity values, eqn (4) was formulated.

$$\begin{aligned} pLD_{50} = {} & 0{\cdot}879(\pm 0{\cdot}236)\,\Delta \log P + 1{\cdot}504(\pm 0{\cdot}567)\Sigma\sigma_I^{ortho} \\ & - 0{\cdot}325(\pm 0{\cdot}186)\Sigma\Delta V_w^{meta} \\ & - 0{\cdot}815(\pm 0{\cdot}250)\,\Delta V_w^{para} \\ & - 2{\cdot}501(\pm 0{\cdot}626)I_{2,6} - 0{\cdot}935(\pm 0{\cdot}239)I_{2,3,5} \\ & + 5{\cdot}946(\pm 0{\cdot}221) \qquad (4) \end{aligned}$$

$$n = 46 \quad s = 0{\cdot}337 \quad r = 0{\cdot}904 \quad F_{6,39} = 29{\cdot}03$$

In this and the following equations, $I_{2,3,5}$ is an indicator variable that takes the value of unity for compounds having either 2,3- or 2,5-disubstitution patterns and two for 2,3,5- and 2,3,4,5-substituted compounds with twice the 2,3- and 2,5-disubstitution patterns, and is otherwise zero. $I_{2,6}$ is another indicator variable that takes the value of unity for compounds having the 2,6-disubstitution pattern and is otherwise zero.

Equation 4 shows that the larvicidal activity of the compounds against rice stem borers increases with increase in the molecular hydrophobicity if substitution patterns are appropriate. Electron-withdrawing effects of *ortho* substituents are favourable to activity. Introduction of *meta* and *para* substituents is unfavourable to activity for steric reasons. The activity is decreased to a varying extent by simultaneous introduction to *ortho* and some other positions: the activity of 2,6-di- and 2,3,5-trisubstituted compounds was about one-hundredth and that of 2,3- and 2,5-disubstituted compounds was about one-tenth that of compounds not possessing these substitution patterns. The activity was not lowered by 2,4-disubstitutions. The intercept value which should be the activity of unsubstituted compound **1** was reasonable. The development of eqn (4) and the degree of collinearity between variables are shown in Tables 3 and 4, respectively. The larvicidal activity values calculated by eqn (4) are listed in Table 1.

TABLE 4
Squared Correlation (r^2) Matrix for Variables Used to Derive Equation (4)

	$\Delta \log P$	$\Sigma\sigma_I^{ortho}$	$\Sigma\Delta V_w^{meta}$	ΔV_w^{para}	$I_{2,6}$
$\Sigma\sigma_I^{ortho}$	0·073				
$\Sigma\Delta V_w^{meta}$	0·223	0·035			
ΔV_w^{para}	0·095	0·080	0·121		
$I_{2,6}$	0·108	0·489	0·048	0·025	
$I_{2,3,5}$	0·138	0·038	0·259	0·034	0·017

4 DISCUSSION

The above quantitative analyses indicate that activity increases linearly with the increase in the molecular hydrophobicity, log *P*, when other factors are separated and substitution patterns are appropriate. In a previous study for larvicidal benzoylphenylureas with the same bioassay system, we have shown that the molecular

hydrophobicity is highly important in determining variations in the larvicidal potency and that quadratic or bilinear terms of log *P* are required to rationalize the hydrophobicity–potency relationship.[36] We have also observed that the larvicidal activity of the benzoylphenylurea derivatives is closely related to their inhibitory effect on the growth of new cuticle in cultured integument fragments excised from rice stem borers. In another study, we have shown that dibenzoylhydrazines as well as 20-hydroxyecdysone inhibit the cuticular growth of cultured integument of rise stem borers[37,38] at a concentration range higher than that required to induce their hormonal activity. In spite of differences in the exact mechanism of action between dibenzoylhydrazines and benzoylphenylureas,[16] the location at which the respective action mechanisms are triggered on the epidermis could be similar. The factors governing the transport process to that location in terms of hydrophobicity would be similar in the two series. However, the largest log *P* value among the dibenzoylhydrazines used to derive eqn (4), 4·67 for compound **53**, was smaller than the log *P* value (5·9) at which the ascending linearity is cut off for the benzoylphenylureas. In fact, the slope of the Δ log *P* term in eqn (4), 0·88, is not far from that of the linear term, 1·18, of the bilinear representation of the hydrophobic effect for 53 benzoylphenylureas.[36]

In eqns (3) and (4), the σ_I^{ortho} term was significant. Neither the replacement of this σ_I parameter with the regular Hammett σ nor the addition of the σ_I^{meta} and σ_I^{para} terms improved the correlations. This indicates that the electronic effect of *ortho* substituents is not operating through bonds, but is of a type of interacting through the field between the *ortho* substituent and the carbamoyl moiety of the side chain. This intramolecular electronic effect could probably operate indirectly on the interaction between the molecule and the receptor, so that the higher the electron-withdrawing field effect of the *ortho* substituents, the higher is the larvicidal activity.

The coefficient of the ΔV_w term for *para* substituents is more negative than for *meta* substituents in eqns (3) and (4). The steric allowance of the receptor to *para* substituents would be lower than that to *meta* substituents. Although the ΔV_w term for *ortho* substituents was not significant for compounds included in eqn (4), their steric effect should be involved because the activity of compounds **10**, **12–14** having bulky *ortho* substituents was too low to measure.

For multi-substituted compounds, the factors dependent on substitution patterns represented by $I_{2,6}$ and $I_{2,3,5}$ were significant in addition to molecular hydrophobicity and position-specific stereo-electronic effects. The $I_{2,6}$ term probably represents a steric factor that participates in distortion of the coplanar conformation of the benzene ring and the side-chain amide moiety. The physicochemical meaning of the $I_{2,3,5}$ term is, however, not clear, but it may be of a steric nature. When one of the *meta* positions is occupied simultaneously with one of the *ortho* positions, the coplanarity might be severely distorted by some repulsive interactions with receptor sites.

Among those tested, compounds **10**, **12–14**, **29**, **32**, **33**, **35**, **49** and **56** showed too low an activity to measure, because of their limited solubility. The low activities of compounds **29**, **32**, **33**, **35**, **49** and **56** were predicted by eqn (4). Although the activity of the SCH_3 compound **14** was measured in the presence of PB, the suppression of the metabolic oxidation may not be complete, giving a less active compound(s) before arriving at the site of action. The unmeasurable activity of *o*-C_6H_5 (compound **10**), *o*-$OCH(CH_3)CH_2CH_3$ (compound **12**), and *o*-$OCH_2C_6H_5$ (compound **13**) derivatives could be due to the unfavourably large size of their substituents as mentioned above. In addition to such latent *ortho*-steric effect, the specific effects assigned to certain multiple substitution patterns are believed to leave us much to explore in future with augmentation of additional compounds and further examination of steric factors.

In conclusion, the higher the hydrophobicity of dibenzoylhydrazines, the higher was the larvicidal activity against the rice stem borer unless substitution patterns were detrimental to the activity. Introducing an *ortho* substituent with an inductively electron-withdrawing property was favourable to the activity. Steric effects on reducing the activity were position-specific. In addition, the activities of compounds having 2,3-, 2,5- and 2,6-disubstitution, but not 2,4-, 3,4- and 3,5-disubstitution patterns were lower than expected from additivity of substituent effects observed for mono-substituted compounds, being partly rationalized by steric effects. The favourable patterns are substitutions at one of the *ortho* positions with hydrophobic, electron-withdrawing and moderate-sized substituents and 3,5-disubstitutions with small-sized hydrophobic substituents.

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REFERENCES

1. Henrick, C. A., Juvenile hormone analogs: Structure–activity relationships. In *Insecticide Mode of Action*, ed. J. R. Coats. Academic Press, New York, 1982, pp. 315–402.
2. Ohsumi, T., Hatakoshi, M., Kishida, H., Matsuo, N., Nakayama, I. & Itaya, N., Oxime ethers: New potent insect growth regulators. *Agric. Biol. Chem.*, **49** (1985) 3197–202.
3. Nakayama, A., Iwamura, H., Niwa, A., Nakagawa, Y. & Fujita, T., Development of insect juvenile hormone active oxime *O*-ethers and carbamates. *J. Agric. Food Chem.*, **33** (1985) 1034–41.

4. Niwa, A., Iwamura, H., Nakagawa, Y. & Fujita, T., Development of (phenoxyphenoxy)- and (benzylphenoxy)-alkanaldoxime *O*-ethers as potent insect juvenile hormone mimics and their quantitative structure–activity relationship. *J. Agric. Food Chem.*, **36** (1988) 378–84.
5. Niwa, A., Iwamura, H., Nakagawa, Y. & Fujita, T., Development of (phenoxyphenoxy)- and (benzylphenoxy)-propyl ethers as potent insect juvenile hormone mimetics. *J. Agric. Food Chem.*, **37** (1989) 462–7.
6. Hayashi, T., Iwamura, H., Nakagawa, Y. & Fujita, T., Development of (4-alkoxyphenoxy)- and (4-alkylphenoxy)-alkanaldoxime *O*-ethers as potent insect juvenile hormone mimics and their structure–activity relationships. *J. Agric. Food Chem.*, **37** (1989) 467–72.
7. Niwa, A., Iwamura, H., Nakagawa, Y. & Fujita, T., Development of *N*,*O*-disubstituted hydroxylamines and *N*,*N*-disubstituted amines as insect juvenile hormone mimetics and the role of the nitrogenous function for activity. *J. Agric. Food Chem.*, **38** (1990) 514–20.
8. Becker, E. & Plagge, E., Über das die Puppariumbildung auslösende Hormon der Fliegen. *Biol. Zbl.*, **59** (1939) 326–41.
9. Karlson, P., Hoffmeister, H., Hummel, H., Hocks, P. & Spiteller, G., Zur Chemie des Ecdysons, VI. Reaktionen des Ecdysonmoleküls. *Chem. Ber.*, **98** (1965) 2394–402.
10. Huber, R. & Hoppe, W., Zur Chemie des Ecdysons, VII. Die Kristall- und Molekülstrukturanalyse des Insekten-verpuppungshormons Ecdyson mit der automatisierten Faltmolekülmethode. *Chem. Ber.*, **98** (1965) 2403–24.
11. Wing, K. D., RH 5849, a nonsteroidal ecdysone agonist: Effects on a *Drosophila* cell line. *Science* (*Washington*), **241** (1988) 467–9.
12. Spindler-Barth, M., Turberg, A. & Spindler, K.-D., On the action of RH 5849, a nonsteroidal ecdysteroid agonist, on a cell line from *Chironomus tentans*. *Arch. Insect Biochem. Physiol.*, **16** (1991) 11–18.
13. Kubo, I., Klocke, J. A. & Asano, S., Effects of ingested phytoecdysteroids on the growth and development of two Lepidopterous larvae. *J. Insect Physiol.*, **29** (1983) 307–16.
14. Chandler, L. D., Pair, S. D. & Harrison, W. E., RH 5992, a new insect growth regulator active against corn earworm and fall armyworm (Lepidoptera: Noctuidae). *J. Econ. Entomol.*, **85** (1992) 1099–103.
15. Darvas, B., Polgár, L., Tag El-Din, M. H., Eröss, K. & Wing, K. D., Developmental disturbances in different insect orders caused by an ecdysteroid agonist, RH 5849. *J. Econ. Entomol.*, **85** (1992) 2107–12.
16. Wing, K. D., Slawecki, R. A. & Carlson, G. R., RH 5849, a nonsteroidal ecdysone agonist: Effects on larval lepidoptera. *Science* (*Washington*), **241** (1988) 470–2.
17. Rohm and Hass Company, Ltd, US Patent 789797 (1985) and 1911177 (1986).
18. Hansch, C. & Fujita, T., $\rho - \sigma - \pi$ Analysis. A method for the correlation of biological activity and chemical structure. *J. Amer. Chem. Soc.*, **86** (1964) 1616–27.
19. Clarke, H. T. & Read, R. R., *o*-Tolunitrile and *p*-tolunitrile. In *Organic Syntheses*, Coll. Vol. 1, ed. H. Gilman. John Wiley & Sons, Inc., New York, 1967, pp. 514–6.
20. Olson, W. T., Hipsher, H. F., Buess, C. M., Goodman, I. A., Hart, I., Lamneck, J. H., Jr. & Gibbons, L. C., The synthesis and purification of ethers. *J. Amer. Chem. Soc.*, **69** (1947) 2451–4.
21. Gilman, H., Langham, W. & Moore, F. W., Some interconversion reactions of organolithium compounds. *J. Amer. Chem. Soc.*, **62** (1940) 2327–35.
22. Kitahara, K., Nakagawa, Y., Nishioka, T. & Fujita, T., Cultured integument of *Chilo suppressalis* as a bioassay system of insect growth regulators. *Agric. Biol. Chem.*, **47** (1983) 1583–9.
23. Nakagawa, Y., Kitahara, K., Nishioka, T., Iwamura, H. & Fujita, T., Quantitative structure–activity studies of benzoylphenylurea larvicides. I. Effect of substituents at aniline moiety against *Chilo suppressalis* Walker. *Pestic. Biochem. Physiol.*, **21** (1984) 309–25
24. Finney, D. J., In *Probit Analysis*. Cambridge Univ. Press, Cambridge, 1952.
25. Goodnight, J. H., In *SAS User's Guide*, ed. J. T. Helwig & K. A. Council. SAS Institute, Inc., Cary, NC, 1979, pp. 357–60.
26. Charton, M., Electrical effect substituent constants for correlation analysis. *Prog. Phys. Org. Chem.*, **13** (1981) 119–251.
27. Bondi, A., van der Waals' volumes and radii. *J. Phys. Chem.*, **68** (1964) 441–51.
28. Verloop, A., The STERIMOL approach: Further development of the method and the new applications. In *Pesticide Chemistry, Human Welfare and Environment*, Vol. 1, ed. J. Miyamato & P. C. Kearney. Pergamon Press, Oxford, 1983, pp. 339–44.
29. Kutter, E. & Hansch, C., Steric parameters in drug design. Monoamine oxidase inhibitors and antihistamines. *J. Med. Chem.*, **12** (1969) 647–52.
30. Hansch, C., Leo, A., Unger, S. T., Kim, K. H., Nikaitani, D. & Lien, E., 'Aromatic' substituent constants for structure–activity correlations. *J. Med. Chem.*, **16** (1973) 1207–16.
31. Nakagawa, S., Okajima, N., Nishimura, K., Fujita, T. & Nakajima, M., Quantitative structure–activity studies of substituted benzyl chrysanthemates. 2. Physicochemical substituent effects and neurophysiological and symptomatic activities against American cockroaches. *Pestic. Biochem. Physiol.*, **17** (1982) 259–70.
32. Sotomatsu, T., Shigemura, M., Murata, Y. & Fujita, T., Octanol/water partition coefficient of *ortho*-substituted aromatic solutes. *J. Pharm. Sci.*, **82** (1993) 776–81.
33. Fujita, T., Substituent effects in the partition coefficient of disubstituted benzenes: Bidirectional Hammett-type relationships. *Prog. Phys. Org. Chem.*, **14** (1983) 75–113.
34. Nakagawa, Y., Izumi, K., Oikawa, N., Sotomatsu, T., Shigemura, M. & Fujita, T., Analysis and prediction of hydrophobicity parameters of substituted acetanilides, benzamides, and related aromatic compounds. *Environ. Toxicol. Chem.*, **11** (1992) 901–16.
35. Hansch, C. & Leo, A. J., CLOGP3 Release 3·54 Medicinal Chemistry Project, Pomona College, Claremont, CA, 1989.
36. Nakagawa, Y., Akagi, T., Iwamura, H. & Fujita, T., Quantitative structure–activity studies of benzoylphenylurea larvicides. V. Substituted pyridyloxyphenyl and related derivatives. *Pestic. Biochem. Physiol.*, **30** (1988) 67–78.
37. Nakagawa, Y., Matsutani, M., Kurihara, N., Nishimura, K. & Fujita, T., Inhibition of N-acetylglucosamine incorporation into the cultured integument of *Chilo suppressalis* by diflubenzuron. *Pestic. Biochem. Physiol.*, **42** (1992) 242–7.
38. Oikawa, N., Nakagawa, Y., Nishimura, K., Soya, Y., Kurihara, N., Ueno, T. & Fujita, T., Enhancement of *N*-acetylglucosamine incorporation into the cultured integument of *Chilo suppressalis* by moulting hormone and dibenzoylhydrazine insecticides. *Pestic. Biochem. Physiol.*, **47** (1993), 165–70.