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Molting hormonal and larvicidal activities of aliphatic acyl analogs of dibenzoylhydrazine insecticides

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*Dibenzoylhydrazines are the nonsteroidal ecdysone agonists. Using comparative molecular field analysis, we previously found that the alkyl side chain of 20-hydroxyecdysone (20E) is three-dimensionally superposable with one of their two aryl moieties. To identify the aryl moiety that is better superposable on the alkyl chain, we synthesized compounds in which one of the two aryl groups of tebufenozide (*N-t-*butyl-*N-*3,5-*dimethylbenzoyl-*N'-*4-ethylbenzoylhydrazine) is replaced by alkyl groups such as* C_4H_9, C_5H_{11}, *and* C_6H_{13}. *The molting hormonal activity of these compounds was measured using cultured integuments prepared from rice stem borers, Chilo suppressalis Walker, in terms of stimulation of incorporation of* N-*acetyl-[*^{14}*C]glucosamine.* N-t-*Butyl-*N-*3,5-dimethylbenzoyl-*N'-*acylhydrazines with a hexanoyl or heptanoyl group were about 20-fold higher than that of 20E, whereas* N-*acyl-*N-t-*butyl-*N'-*4-ethylbenzoylhydrazines with a hexanoyl or heptanoyl group were much weaker than 20E. Their larvicidal activity was also measured against rice stem borers. The former series of compounds were much more active than the other series as well as 20E. Thus, the benzoyl moiety of dibenzoylhydrazines, which is bound to the secondary nitrogen atom (-NH-), is replaceable by aliphatic acyl groups without greatly affecting the biological activities.* (*Steroids* **62**:638–642, 1997) © *1997 by Elsevier Science Inc.*

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Introduction

N,N'-Dibenzoyl-*N-t*-butylhydrazines (**I**), such as RH 5849 (**I**: X_n=Y_n=H) and RH 5992 (**I**: X_n=3,5-Me_2, Y_n=4-Et; tebufenozide), are well known as nonsteroidal ecdysone agonists, which induce prematurely abnormal and, ultimately, lethal larval molting.[1] Certain of these compounds, which contain various substituent groups on the benzene rings, such as tebufenozide, are used commercially in some countries. Despite large differences in the chemical structures of dibenzoylhydrazines, compared with the natural steroidal molting hormone, 20-hydroxyecdysone (20E; **II**), the dibenzoylhydrazines (**I**) have been shown to bind to the ecdysone receptor (EcR) in a cell free system.[2,3] Recently, the EcR gene was cloned, and the binding region of EcR with ligands was examined to better understand the mode of action of these compounds at the molecular level.[4–7]

In earlier work, we reported the synthesis and characterization of *N,N'*-dibenzoyl-*N-t*-butylhydrazines (**I**) having a variety of substituents on the two benzene rings (**I**, A and B) and measurements of the in vivo and in vitro activities against the rice stem borer (*Chilo suppressalis* Walker).[8–10] The results showed that the activities are enhanced with an increase in molecular hydrophobicity and that there are favorable electronic effects and unfavorable steric effects of substituents on the benzene rings which contribute to the activity. We also measured the in vitro activity of five 20E analogs[11] and quantitatively analyzed the structure–activity relationship for the combined set of ecdysones and dibenzoylhydrazines[12] using comparative molecular field analy-

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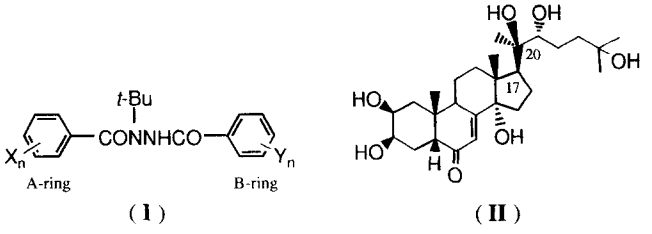

(I) (II)

sis (CoMFA).[13] The CoMFA results suggested that one of the benzoyl moieties of the dibenzoylhydrazines (**I**) is superposable on the alkyl side chain at the 17-position of 20E (**II**), although the precise benzoyl group could not be identified.

In this study, we wish to report the synthesis of some analogs of tebufenozide, where either the A- or B-ring is replaced with an alkyl chain (C_4–C_6), and measurement of their molting hormonal activity in the cultured integument system and their larvicidal activity. The results show that the benzoyl moiety of the dibenzoylhydrazines, which is bound to the secondary nitrogen atom (-NH-), is replaceable by aliphatic acyl groups without greatly affecting biological activities, whereas the replacement of the other benzoyl moiety with aliphatic acyl groups results in a drastic decrease in both in vitro and in vivo activities.

Experimental

Chemicals

The tebufenozide used in this study was the same sample as reported in a prior study.[9] Other acylhydrazines (**III** in Figure 1) listed in Table 1 were synthesized using conventional procedures,[8,9,14] as shown in Figure 1. The structures were confirmed by NMR spectroscopy and elemental analyses. The analytical values for C, H, and N agreed with the calculated values within ±0.3%. The intermediate and final compounds were purified by distillation, recrystallization, or column chromatography on a Wakogel C-200. ^{1}H-NMR spectra were recorded on a Brucker AC-300 NMR spectrometer at 300 MHz in deuteriochloroform ($CDCl_3$) with tetramethylsilane as the internal standard. Melting points of the compounds listed in Table 1 were determined on a Yanako melting point apparatus and are uncorrected. 20E was purchased from Sigma Chemical Co. (St. Louis, Missouri, USA) and *N*-acetyl-[1-^{14}C]glucosamine ([^{14}C]GluNAc, 58.7 mCi/mmol) was obtained from Amersham International plc (Buckinghamshire, United Kingdom). Other chemicals were obtained from Wako Pure Chemical Ind., Ltd. (Osaka, Japan) and Nacalai Tesque, Inc. (Kyoto, Japan).

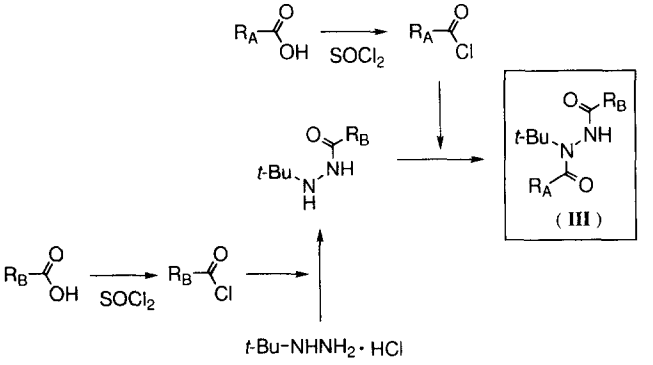

Figure 1 Scheme for the synthesis of acyl benzoylhydrazines.

***N*-*t*-Butyl-*N*-3,5-dimethylbenzoyl-*N'*-*n*-heptanoylhydrazine.** *n*-Heptanoic acid (5.0 g; 43.0 mmol) was refluxed with thionyl chloride (6.85 g; 57.6 mmol) at 80°C for 5 h. After evaporating the excess thionyl chloride under reduced pressure, *n*-heptanoyl chloride (3.26 g; 24.2 mmol) was purified by distillation (b.p. 150°C/760 mmHg; yield 56.3%). 3,5-Dimethylbenzoyl chloride (4.77 g; 28.2 mmol; b.p. 88°C/8 mmHg) was prepared by reacting 3,5-dimethylbenzoic acid (5.03 g; 33.5 mmol) and thionyl chloride (6.85 g; 57.6 mmol) as described above (yield 84.2%). *n*-Heptanoyl chloride (1.1 g; 8.17 mmol) and a 50% aqueous

Table 1 Molting hormonal and larvicidal activities of test compounds and their melting points

Compounds (III)[a]			Biological activities		
No.	R_A	R_B	pEC_{50} (M)	pLD_{50}[b] (mmol/larva)	m.p. (°C)
1	Ph(3,5-Me_2) (tebufenozide)	Ph(4-Et)	8.94	7.32[c]	194–195[c]
2	Ph(3,5-Me_2)	*n*-C_4H_9	7.10	6.31	91–92
3	Ph(3,5-Me_2)	*n*-C_5H_{11}	8.05	6.08	144–145
4	Ph(3,5-Me_2)	*i*-C_5H_{11}[d]	7.97	6.75	136–137
5	Ph(3,5-Me_2)	*n*-C_6H_{13}	8.13	5.93	115–116
6	Ph(3,5-Me_2)	*i*-C_6H_{13}[e]	7.96	5.57	161–162
7	*n*-C_5H_{11}	Ph(4-Et)	5.09	<4.30 (6.7%)	112–113
8	*i*-C_5-H_{11}[d]	Ph(4-Et)	5.34	<4.30 (22.2%)	129–130
9	*n*-C_6H_{13}	Ph(4-Et)	5.17	<4.30 (16.7%)	140–141
10	*i*-C_6H_{13}[e]	Ph(4-Et)	5.04	<4.30 (0%)	156–157
11	20E		6.75[f]	<4.28 (0%)	–

[a]See Figure 1.
[b]The values in parentheses are % mortality at the maximum dose indicated.
[c]From Ref. 9.
[d]$(CH_3)_2CHCH_2CH_2$-.
[e]$(CH_3)_2CHCH_2CH_2CH_2$-.
[f]From Refs. 11 and 12.

solution of sodium hydroxide (2.0 g; 25.0 mmol) were simultaneously added dropwise to a suspension of *t*-butylhydrazine hydrochloride (0.85 g; 6.85 mmol) in 75 mL of 1,4-dioxane/water (2:1, v/v) with stirring on an ice bath. After stirring for 2 days at room temperature, dioxane was removed under reduced pressure and the residue was extracted with ether (Et_2O). The organic phase was washed once with 1 N NaOH and brine and then dried over anhydrous magnesium sulfate. The residue obtained by evaporation of the Et_2O under reduced pressure was purified by silica-gel column chromatography with hexane/ethyl acetate (1:1, v/v) to afford *N*-*t*-butyl-*N'*-*n*-heptanoylhydrazine (0.51 g; 2.74 mmol) as a yellowish oil (yield 40.0%). 3,5-Dimethylbenzoyl chloride (0.35 g; 2.08 mmol) and 1 N NaOH (4.0 mL) were simultaneously added dropwise to *N*-*t*-butyl-*N'*-n-heptanoylhydrazine (0.43 g; 2.31 mmol) dissolved in Et_2O (5.0 mL), and stirred for 2 days at room temperature. The reaction mixture was diluted with additional Et_2O (50 mL) and washed successively with 1 N NaOH, 1 N hydrochloric acid, and brine. The organic layer was dried over anhydrous magnesium sulfate, and after concentration, the residue was percolated through a silica-gel column and eluted with hexane/ethyl acetate (2:1, v/v). The residue obtained after removing the solvent under reduced pressure was recrystallized from hexane and Et_2O to give *N*-*t*-butyl-*N*-(3,5-dimethylbenzoyl)-*N'*-*n*-heptanoylhydrazine (0.68 g; 2.14 mmol; yield 92.6%). ^1H NMR ($CDCl_3$): δ (ppm): 1.2 (11H, m), 1.5 (9H, s), 1.8 (2H, br), 2.2 (6H, s), 7.0 (3H, s), 8.0 (1H, br). The other compounds were synthesized using similar procedures. The melting points of these compounds are listed in Table 1.

Molecular modeling

All computations were performed with the molecular modeling software package SYBYL, version 6.2. The conformation of compound **3** was deduced from the x-ray diffraction structure of *N*-*t*-butyl-*N,N'*-dibenzoylhydrazine (RH 5849), which was reported previously,[12] and was fully optimized by the semi-empirical molecular orbital method, PM3, in the program package MOPAC 5.0. The structure of 20E was also constructed based on its reported x-ray structure and was optimized by PM3.

Bioassays

The molting hormonal activity of the test compounds was evaluated using the cultured integument system as reported earlier.[15] Briefly, integument fragments excised from the diapause larvae of rice stem borers were floated on the medium containing the test compounds at various concentrations and cultured at 25°C for 24 h. For each set of experiments, two groups of integument fragments were treated with 0.1% dimethyl sulfoxide (DMSO) and 20E (1.0 ppm) as a control and a positive standard, respectively. These integument fragments were transferred to the fresh medium containing [^{14}C]GluNAc (ca. 6000–8000 dpm/μL) and cultured for 72 h at 25°C. The integument fragments were then washed with distilled water, and the radioactivity incorporated into the fragments was measured in Aquasol II (NEN DuPont, Boston, Massachusetts, USA) with a liquid scintillation counter.

The larvicidal activity of the test compounds was determined against the rice stem borer as described previously.[8,9] Various doses of each compound in DMSO (0.5 μL) were topically applied to the dorsal area of 20 third-instar larvae. After rearing for 7 days on a diet containing piperonyl butoxide (100 μM) at 28°C, the mortality was observed. A dose-response relationship for the larvicidal effect of compound **5** was constructed and is shown in Figure 2. The activity in terms of pLD_{50}, the log value of the reciprocal of the dose (mmol/larva) required to kill half the larvae, was estimated by probit transformation[16,17] and is listed in Table 1.

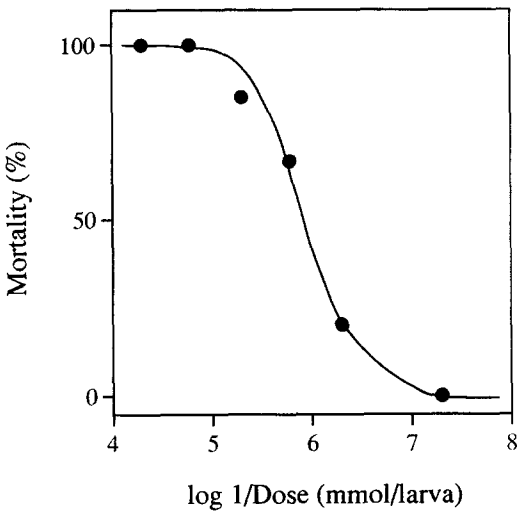

Figure 2 Dose–response relationship for the larvicidal activity of compound **5**.

Results

Molting hormonal activity

The concentration-response relationship of compound **5** in terms of the incorporation of [^{14}C]GluNAc into the cultured integument is depicted in Figure 3. The incorporation was stimulated by the compound up to approximately 2×10^{-8} M. The stimulation was reduced to the control level by increasing the concentration. This trend was generally observed with highly or moderately active compounds. By taking the lower value of the control and the minimum in the increasing phase of the concentration–response relationship, and the higher value of the maximum in the concentration–response relationship and a value with 20E as 0 and 100%, respectively, the relationship for compound **5** was

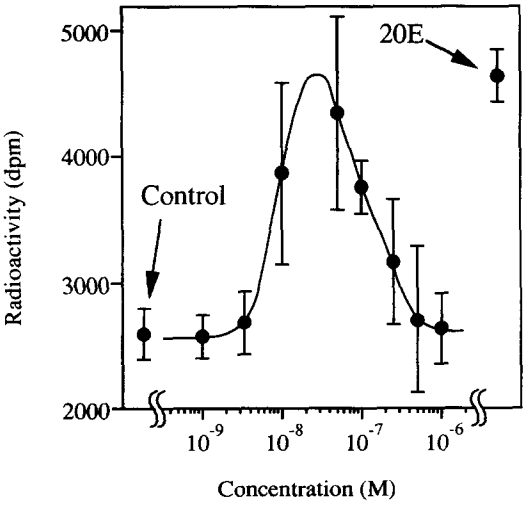

Figure 3 Concentration–response relationship of compound **5** in enhancing [^{14}C]GluNAc incorporation into the cultured integument. The vertical bars show the standard deviation of three runs. The abscissa is drawn on a logarithmic scale.

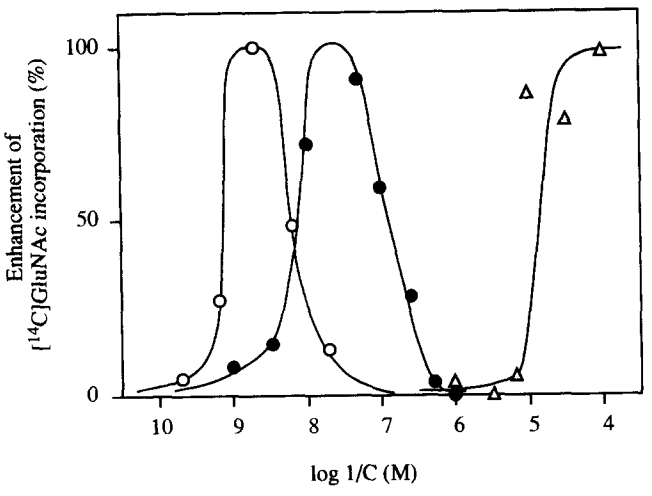

Figure 4 Concentration–response curves of compounds **1** (○), **5** (●) and **7** (△) in enhancing [^{14}C]GluNAc incorporation into the cultured integument. The enhancement is expressed as a percentage by taking the minimum as 0% and the maximum as 100% for each compound. For compound **5**, the positive standard with 20E was taken as 100%. The pEC_{50} value was evaluated from the ascending phase of each curve.

reconstructed as shown in Figure 4, along with those for compounds **1** and **7**. From these curves, the half-effective concentration, EC_{50}(M), at the increasing phase was estimated by probit analysis.[16,17] The reciprocal logarithm of EC_{50}, pEC_{50}, listed in Table 1 was used as an index of the molting hormonal activity.

Compounds **2–6** were more potent than 20E. Among them, compounds **3–6** in which the 4-ethylphenyl group of tebufenozide was replaced with C_5 or C_6 alkyl chains, were approximately 20-fold more potent than 20E, and 1/10 that of tebufenozide. Compound **2** with a shorter alkyl chain was less potent than compounds **3–6** by a factor of 10. The activity of compounds **7–10** was very weak and about 1/1000 of the corresponding aliphatic-acyl compounds **3–5**.

Larvicidal activity

Among the aliphatic acyl analogs **2–10**, compound **4** was the most active. It was weaker than tebufenozide by a factor of only four and was two times more potent than RH 5849 (X_n=Y_n=H in **I**: pLD_{50} = 6.29[8]). Although the in vitro activity of compound **2** with a C_4 alkyl chain was lower than compounds **3–6** with a C_5 or C_6 alkyl chain by a factor of 10, its larvicidal activity was not so much different from others. The activity of the other set of compounds **7–10**, in which the 3,5-dimethylphenyl group of tebufenozide was replaced with a C_5 or C_6 alkyl chain, was too low to determine their pLD_{50} values. 20E was larvicidally inactive under the test conditions.

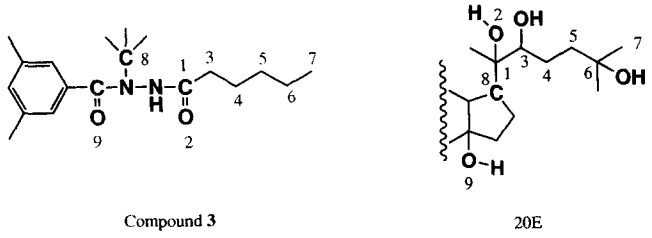

Figure 5 Procedure for the superposition between compound **3** and 20E. These two compounds were superposed on each other so as to minimize the sum of the squares of distances between the key atoms of the molecules with the same number.

Discussion

In an earlier paper, we reported a quantitative analysis of the molting hormonal activity of dibenzoylhydrazines and ecdysone analogs conducted by CoMFA.[12] In CoMFA, compounds should be superposed first reasonably based on their structural similarity. Skeletons of the two series of compounds are very different, so there are a number of ways to superpose on each other. Among them, we chose two ways in which each of the A- and B-rings of the dibenzoylhydrazines is superposed on the side chain of ecdysone analogs. To the extent that the CoMFA correlation statistics are concerned, these two superpositions are nearly equivalent.[12] Results listed in Table 1 indicate that the B-ring moiety but not the A-ring of the dibenzoylhydrazines is replaceable with alkyl chains without greatly affecting the biological activities. This may suggest that the B-ring moiety of the dibenzoylhydrazines has a role similar to that of the alkyl side chain of ecdysones to give potent activities. In fact,

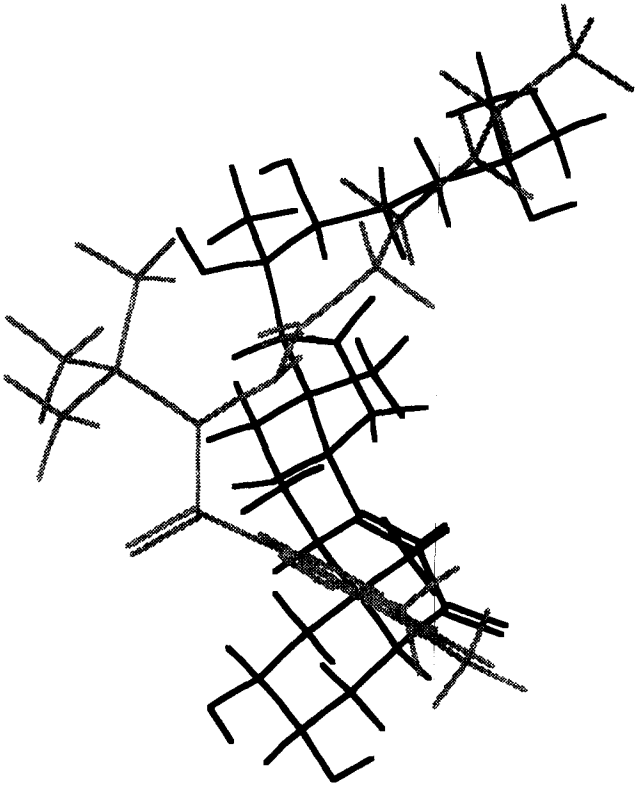

Figure 6 Superposition of compound **3** (shaded line) on 20E (solid line).

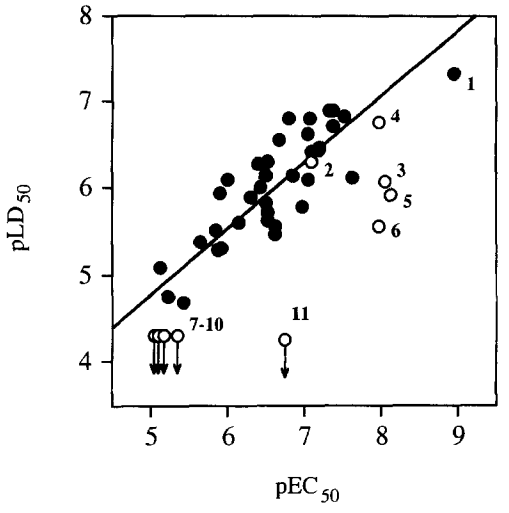

Figure 7 Relationship between molting hormonal and larvicidal activities against *C. suppressalis*. The closed circles represent substituted dibenzoylhydrazines (Ref. 8–10). Data for open circles were taken from Table 1.

based on the procedure shown in Figure 5, compound **3** gave a fairly good superposition on 20E (Figure 6). In addition to steric factors, quantitative examinations based on electronic and hydrophobic interactions are required to obtain reasonable results in the molecular modeling study.

We previously reported that there is a linear relationship between larvicidal and hormonal activities for a set of dibenzoylhydrazines.[10,18] Among compounds **2–6**, which had definitive larvicidal activity values, compounds **2** and **4** fell on the relationship as shown in Figure 7. The larvicidal activity values of compounds **3**, **5**, and **6**, as well as 20E, were, however, lower than those predicted from their in vitro activity. A plausible explanation for these negative deviations is that they undergo detoxification and are converted into inactive metabolites during the incubation period.

In summary, aliphatic acyl analogs of dibenzoylhydrazines at the B-ring moiety were much more potent than the other series of compounds in both larvicidal and hormonal activities. Particularly, the former series of compounds were more potent than 20E in the hormonal activity. Quantitative three-dimensional structure–activity analyses for ecdysones and dibenzoylhydrazines including the present compounds are in progress to delineate a more comprehensive understanding, which, in turn, will allow the design of new compounds with this type of activity.

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