Molecular Modeling Study on the Structure–Activity Relationship of Substituted Dibenzoyl-1-*tert*-butylhydrazines and Their Structural Similarity to 20-Hydroxyecdysone

Xuhong Qian

Institute of Pesticides and Pharmaceuticals, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, China

Several possible three-dimensional conformations of substituted dibenzoyl-1-*tert*-butylhydrazine (SBH) were established with the help of a molecular modeling method. It was found that there was a good parabolic relationship between larvicidal activity (pLD_{50} or pLD_{50}^{-1}) and the nearest distance *r* of the oxygen atomic center of carbonyl group A to the atomic center connecting with benzene ring B of substituents in conformation I of SBH. Molecular mechanics calculations revealed that SBH and 20-hydroxyecdysone have several similarities in the π-electronic conjugated area, flexible alkyl group and strong negative electronic center near the conjugated area; therefore, SBH might mimic the binding region of ecdysone.

Keywords: *Substituted dibenzoyl-1-tert-butylhydrazine; 20-hydroxyecdysone; quantitative structure–activity relationship; molecular modeling*

INTRODUCTION

Juvenile and molting hormones regulate insect metamorphosis and development, and new generation insecticides that regulate the action of these hormones are specific to insects and scarcely toxic to mammals. It is well-known that the steroid hormone 20-hydroxyecdysone (20-HE) controls insect molting, especially for the production of new cuticle. Recently, nonsteroidal 1,2-dibenzoyl-1-*tert*-butylhydrazine and its congeners (SBH), as a new class of insecticides having totally different structures, were found to mimic the action of 20-HE to activate the ecdysone receptor (Wing et al., 1988a; Spindler-Barth et al., 1991), leading to lethal premature molting (Kubo et al., 1983; Chandler et al., 1992; Darvas et al., 1992); the former was found to be more potent than the latter (Wing et al., 1988b). Some researchers have studied the synthesis and quantitative structure–activity relationship of several substituted 1,2-dibenzoyl-1-*tert*-butylhydrazines (Oikawa et al., 1994a,b; Mohammed-Ali et al., 1995); it was found that the effects of substituents on activity were a very complicated case, as various effects, e.g. molecular hydrophobicity and steric and electronic factors, seemed to overlap (Oikawa et al., 1994a). The postulated additivity of effects of substituents on activity could not explain why the activities of 2,3-, 2,5-, and 2,6-disubstituted and 2,3,5- and 2,3,4-trisubstituted compounds were lower than those of 2,4-, 3,4-, and 3,5-disubstituted derivatives (Oikawa et al., 1994a).

In this paper, we report molecular modeling studies of the three-dimensional structures of substituted 1,2-dibenzoyl-1-*tert*-butylhydrazines, their structure–activity relationships, and the structural similarity between them and 20-hydroxyecdysone (Figure 1).

MATERIALS AND METHODS

Larvicidal activities [LD_{50} (millimoles per insect)] of substituted dibenzoyl-1-*tert*-butylhydrazines against the rice stem borer were taken from the literature (Oikawa et al., 1994a).

The procedure used for the molecular modeling study was PCMODEL, (fourth edition, June 1990) of Serena Software (Bloomington, IN). The iterative energy minimization of molecular structure was carried out by using the MMX-Minimize program (Mohammed-Ali et al., 1995). All conformations, energies (*E*, kcal/mol), dihedral angles (deg), and bond lengths (Å) were obtained after running MMX-Minimize. The MMX force field embodies much work to extend and improve upon the MM2 force field. The starting point for MMX was the MM2(77) program. To this was added the VESCF π routines from MMPI and the concept of generalized parameters. The goal of this work was to be able to treat more compounds; for example, such functional groups as radicals, cations, and anions are not handled in MM2.

Since minimization is quite time-consuming with large molecules, it is often best to minimize the structure without added hydrogens to obtain the approximate three-dimensional structure and then to perform final minimization. Usually the three-dimensional structure of dibenzoyl-1-*tert*-butylhydrazine (RH 5849) in a conformation was first established by using the MMX-M program when the iteration of energy minimization stopped and the related index was output; then the structures of other compounds in the same conformation were similarly established using the three-dimensional structure of dibenzoyl-1-*tert*-butylhydrazine as a template.

Compounds with an NO_2 group were not included as NO_2 parameters are not available in this program. The nearest distance *r* from the oxygen atomic center at carbonyl group A to the atomic center connecting with benzene ring B of substituents was written as *r* (Å) (see Figure 1).

RESULTS AND DISCUSSION

Conformation of SBH. Many possible three-dimensional molecular conformations of substituted 1,2-dibenzoyl-1-*tert*-butylhydrazines and their energies were studied with using the PCMODEL method. The four structural conformations with the lowest energies are shown in Figure 2. These conformations have some common features, that is, the atoms of each of the two phenyl groups are coplanar; however, the two carbonyl groups to which they are bonded are not coplanar with the phenyl rings. The energies of RH 5849 (in kilocalories per mole) of these conformations were in the following order: I (32.73) < III (46.16) < II (44.28) < IV (53.79). In addition, these conformations have similar electronic charge distributions.

For substituted dibenzoyl-1-*tert*-hydrazines, conformation I had the lowest energy. Here, the bulky *tert*-butyl group causes the phenyl group to be directed away

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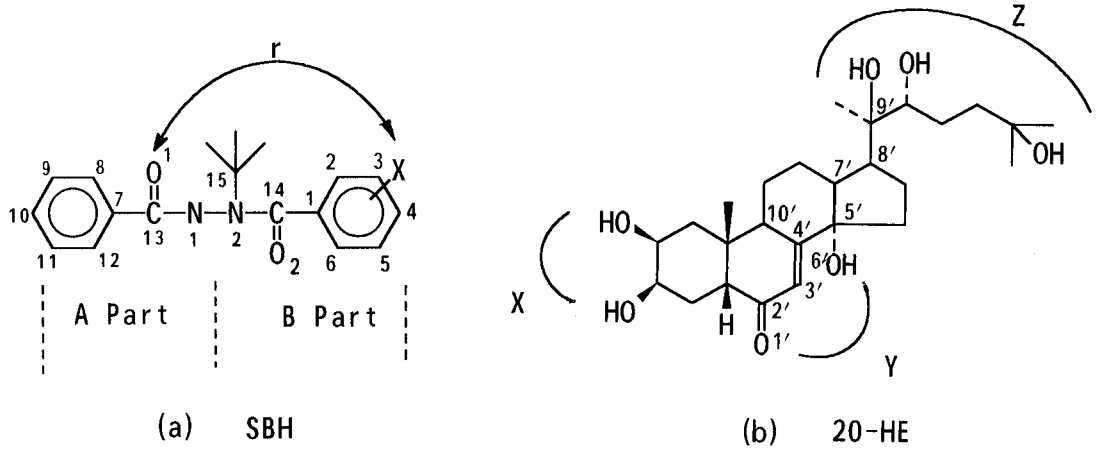

Figure 1. (a) Structure of 1-(substituted benzoyl)-2-benzoyl-1-*tert*-butylhydrazine and demonstration of distance *r*. (b) Structure of 20-hydroxyecdysone and its three binding regions, X, Y, and Z.

Table 1. Some Bond Lengths, Angles, and Dihedral Angles of RH 5849

	conformation	X-ray structure	
	I	A	B
N(2)−N(1)−C(13) (deg)	124.01	120.2(3)	120.0(3)
N(1)−N(2)−C(14) (deg)	120.49	116.7(3)	115.2(3)
N(1)−N(2)−C(15) (deg)	113.56	117.6(2)	116.8(2)
C(14)−N(2)−C(15) (deg)	125.93	123.3(3)	123.0(3)
C(2)−C(1)−C(14)−O(2) (deg)	124.50	114.3(4)	122.0(4)
C(12)−C(7)−C(13)−O(1) (deg)	148.01	−149.6(4)	−153.8(4)
C(13)−N(1)−N(2)−C(14) (deg)	−46.10	−71.8	−58.1
N(2)−C(15) (Å)	1.486	1.506(4)	1.507(4)
O(1)−C(13) (Å)	1.227	1.219(5)	1.211(5)
O(2)−C(14) (Å)	1.214	1.226(5)	1.230(5)
N(1)−N(2) (Å)	1.499	1.394(4)	1.394(4)
dihedral angle of the two phenyl rings (deg)	66.60	66.3(2)	60.8(2)

and the two amide functions adopt different planar conformations. The amide bearing the N−H has the *s-cis* conformation O(1)−C(13)−N(1)−N(2), whereas the amide bearing the *N-tert*-butyl group has the *s-trans* conformation O(2)−C(14)−N(2)−N(1). These results were similar to that obtained for the X-ray structures in which there are two molecules in very similar conformations A and B in the asymmetric unit (Chan et al., 1990). Comparison of the data for both analyses is given in Table 1.

Structure−Activity Relationship. Generally speaking, hydrophobicity and steric and electronic properties are major factors affecting biological activities of compounds. For a series of biologically active compounds with common structural features, the change of the molecular shape or three-dimensional molecular conformation caused by various substituents might be the most important factor, or only factor, in determining the potency level of biological activity. This follows because a drug's major electronic character has been fixed in the primary structure or in the common structural part, and molecular shape is the only significant variable.

Some researchers believed that the effects of substituents at the benzoyl moiety on activity were more complicated, and it was difficult to explain the difference in activity between mono- or disubstituted and multisubstituted with multivariables and one unified QSAR equation (Oikawa et al., 1994a). Other researchers believed that biological activity correlates mainly with π value (lipophilic character) and L value (steric effect); high π and low L values would increase the activity, and, of course, the involvement of π values will improve the correlation coefficient (Mohammed-Ali et al., 1995).

Here, we propose to describe the change of the activity relationship with one variable corresponding to molecular shape, rather than multivariables, e.g. hydrophobicity and electronic and steric factors.

Suppose that conformation I with the lowest energy stands for the real three-dimensional structure of substituted dibenzoyl-1-*tert*-butylhydrazine, i.e. the structure that activates the receptor. Then, there should be a correlation between structural index and activity.

Several distances, angles, and dihedral angles among atoms, groups, or rings in conformation I were calculated. There was a relationship only between activity and the nearest distance *r* from the oxygen atomic center of carbonyl group A to an atomic center of substituents on benzene ring B (Table 2). However, for different types of substituents, e.g. halogen, alkyl, and alkoxyl (alkylthioxy), relationships were different for each substituent, although their general trends were similar.

When substituents were halogens, whatever the number or position of substituents, there was a very good parabolic relationship between the distances *r* and activities (eqs 1−3 in Table 3), and an optimum area existed for the distances *r* corresponding to the higher activities. When the distance *r* is too short or too far, activities decreased; for multisubstituted compounds the effect of a substituent near the oxygen atom at carbonyl group A was the most significant. This effect was easily understood from the role of ortho-halogen groups as substituents. Thus, the change of molecular shape resulting from a substituent's steric hindrance might be the key factor affecting the interaction between a compound and the receptor (see Figure 3a). The statistical results are given in Table 3.

Similar results were obtained with alkyl (including phenyl, CF_3, and NC) groups as substituents, in which a carbon atom was directly connected to benzene ring B (see Figure 3b), or alkoxy (including alkthioxy) groups as substituents (see Figure 3c), in which an oxygen or sulfur atom was directly connected to benzene ring B.

However, for substituted dibenzoyl-1-*tert*-butylhydrazines in conformations II−IV, we could not find any obvious structure−activity relationship. For example, in the case of halogens as substituents at benzene ring B we also checked the change of larvicidal activity corresponding to the nearest distance *r* in conformations II−IV with higher energies; their correlated relation-

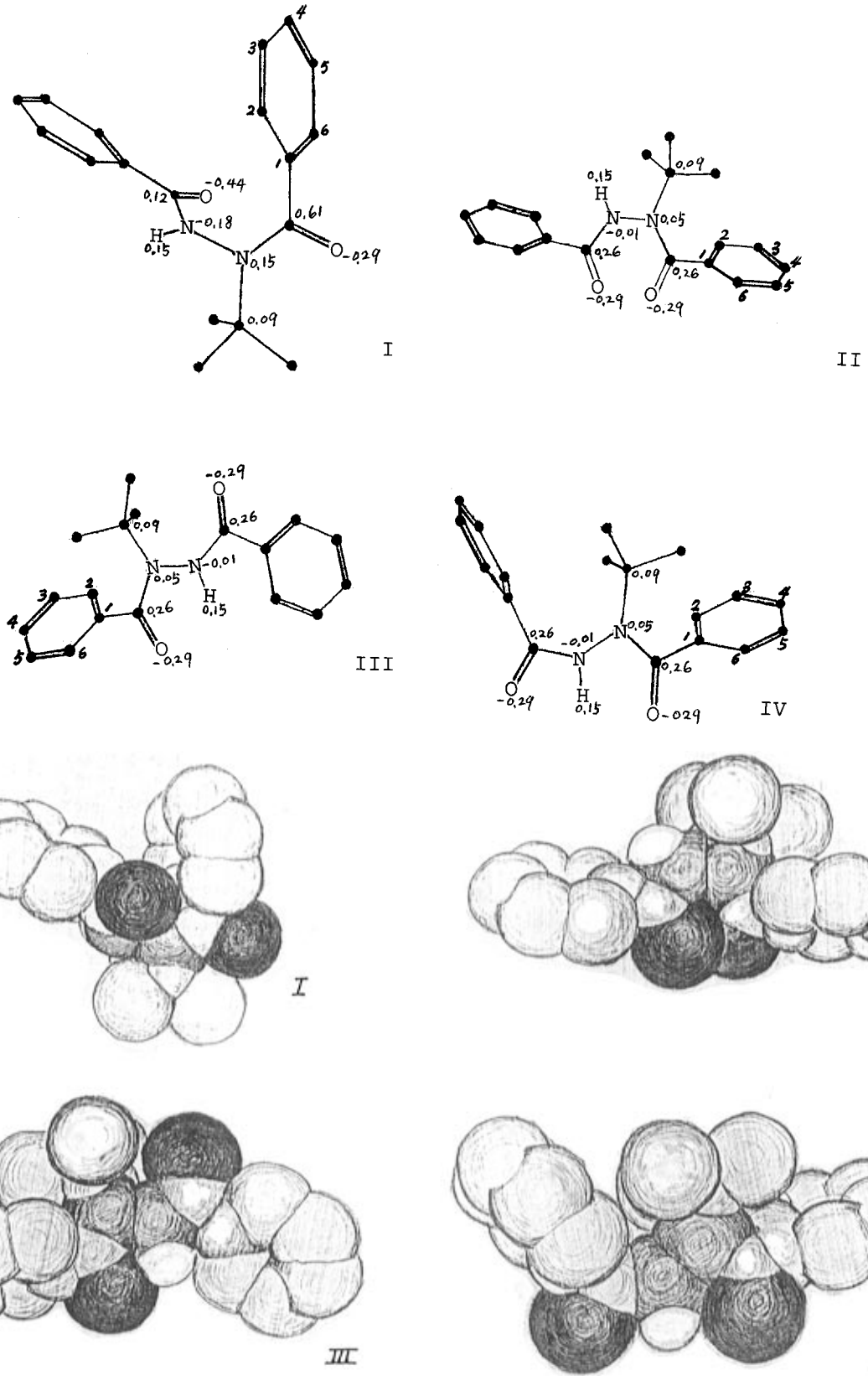

Figure 2. Three-dimensional structure, distribution of electronic charge (top) and three-dimensional van der Waals radii surface (bottom) of RH 5849 in conformations I−IV.

ships were very poor (eqs 4−6 in Table 3; Figure 4), and no other apparent correlated relationship was obtained.

The above results suggested that the distance index *r* in conformation I might reflect important structural features of substituted dibenzoyl-1-*tert*-butylhydrazines when they activated the receptor, and their differences in correlated relationship for halogen, alkyl, and alkoxy as substituents might be due to the changes of the nature of the atom connected directly with benzene ring B.

Possible Action Model of SBH and Comparison of the Structure of SBH with That of 20-HE. The above analyses revealed that there were three possible

Table 2. Distances *r* in Conformation I and Larvicidal Activities of Substituted Dibenzoyl-1-*tert*-butylhydrazines

substituent X	position at conformation I	distance *r*	energy	pLD_{50}
2-F		4.010	17.84	6.24(±0.16)
2-Cl		4.480	20.87	6.83(±0.36)
2-Br		4.679	22.79	6.88(±0.23)
2-I		4.895	23.88	6.99(±0.11)
3-F		5.027	31.58	6.31(±0.12)
3-Cl		5.307	31.81	6.45(±0.06)
3-Br		5.395	31.85	6.49(±0.15)
3-I		5.548	31.61	6.58
4-F		5.207	32.51	6.44
4-Cl		5.547	32.81	6.55(±0.11)
4-Br		5.685	32.91	5.94
4-I		5.857	32.69	5.51(±0.16)
2,3-Cl_2		4.458	25.06	6.33(±0.04)
2,4-Cl_2		4.459	17.10	7.01(±0.05)
2,5-Cl_2		4.453	20.99	6.36(±0.03)
2,6-F_2		3.329	−0.99	5.02(±0.17)
2-F,6-Cl		3.422	2.69	5.10
2,6-Cl_2		3.399	6.40	4.52
3,4-Cl_2		5.219	36.21	6.78(±0.25)
3,5-Cl_2		4.720	27.42	7.07(±0.09)
2,3,4-Cl_3		4.406	26.03	6.17(±0.16)
2,3,4,5-F_4		3.902	23.06	5.38(±0.03)
2,3,4,5,6-F_5		3.282	5.01	<4.48
2-CH_3		5.189	31.95	5.82(±0.16)
2-CF_3		4.614	63.12	6.90(±0.23)
2-C_2H_5		4.720	32.43	6.03
2-C_6H_5		4.367	41.16	<5.00
2-CH_3, 3-Cl		4.410	31.68	5.76
2,3-$(CH_3)_2$		4.433	30.84	4.54(±0.01)
2,4-$(CH_3)_2$		4.456	30.19	4.92
2,5-$(CH_3)_2$		4.475	30.17	5.64(±0.09)
3-CH_3		5.189	31.95	6.10(±0.30)
3-CF_3		5.110	64.92	6.02(±0.02)
3-CF_3, 2,5-Cl_2		4.286	40.62	5.28
3-CN	5	4.367	36.03	4.68(±0.06)
3,4-$(CH_3)_2$		5.203	32.20	5.52(±0.02)
3,5-$(CH_3)_2$		4.581	31.34	6.43(±0.19)
4-CH_3		5.408	32.37	5.61(±0.02)
4-CF_3		5.352	65.24	5.58
4-CN		5.119	36.38	5.09
4-$C(CH_3)_3$		5.359	36.86	<4.48
4-C_6H_5		5.417	45.19	<4.48
2-OCH_3		4.129	31.63	6.14(±0.06)
2-$OCH(CH_3)CH_2CH_3$	6	3.322	36.66	<4.00
2-$OCH_2C_6H_5$	6	3.432	43.82	<4.00
2-OCH_3, 5-C_3H_7		4.080	33.45	5.21(±0.11)
2-OCH_3, 3,5-$(CH_3)_2$		4.175	33.25	4.72
2-SCH_3		4.627	30.21	<5.00
3-OCH_3		5.089	35.29	5.89(±0.23)
3,4-$(OCH_3)_2$		5.122	40.53	<5.00
4-OCH_3		5.334	36.16	4.76
4-$O(CH_2)_3C_6H_5$		5.507	47.14	<4.48

Table 3. Regression Equations for Biological Activity of Substituted Dibenzoyl-1-*tert*-butylhydrazine with Halogen Substituents Connecting with Benzene B ($n = 23$)

eq		R^2	*s*
1	$pLD_{50}^{-1} = 0.807 - 0.271r + 0.0278r^2$	0.893	0.008252
2	$\ln pLD_{50} = -1.84 + 1.54r - 0.159r^2$	0.882	0.04889
3	$pLD_{50} = -14.8 + 8.88r - 0.917r^2$	0.864	0.3002
4	$pLD_{50}^{-1} = 0.272 - 0.0488r + 0.00345r^2$	0.141	0.02342
5	$pLD_{50}^{-1} = 0.370 - 0.0538r + 0.00335r^2$	0.192	0.02289
6	$pLD_{50}^{-1} = 0.393 - 0.0587r + 0.00357r^2$	0.185	0.02246

factors governing the activities of substituted dibenzoyl-1-*tert*-butylhydrazine to receptor: (1) the oxygen atom with negative charge at carbonyl group A could play a very important role; (2) there was a suitable distance between that oxygen and the connecting atom of the substituent; (3) the molecular shape affected the activity greatly, and conformation I might be believed an acceptable three-dimensional conformation for substituted

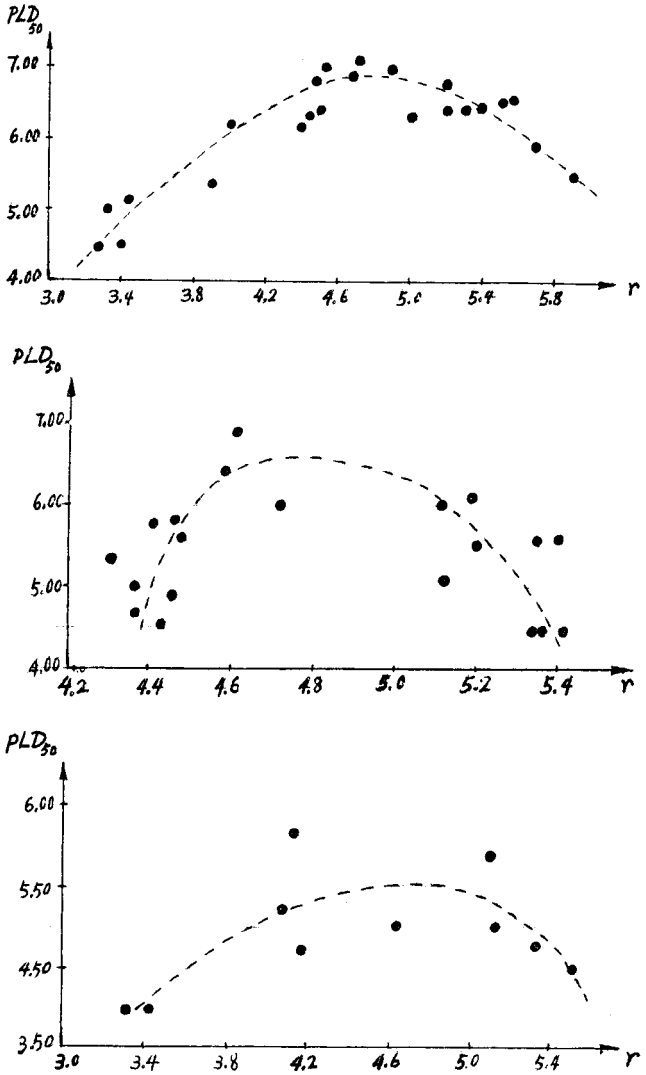

Figure 3. Correlation between the nearest distances *r* in conformation I and larvicidal activities pLD_{50} of substituted dibenzoyl-1-*tert*-butylhydrazines: (a, top) for substituents with halogen as the connecting atom with benzene B; (b, middle) for substituents with carbon as the connecting atom with benzene B; (c, bottom) for substituents with oxygen (sulfur) as the connecting atom.

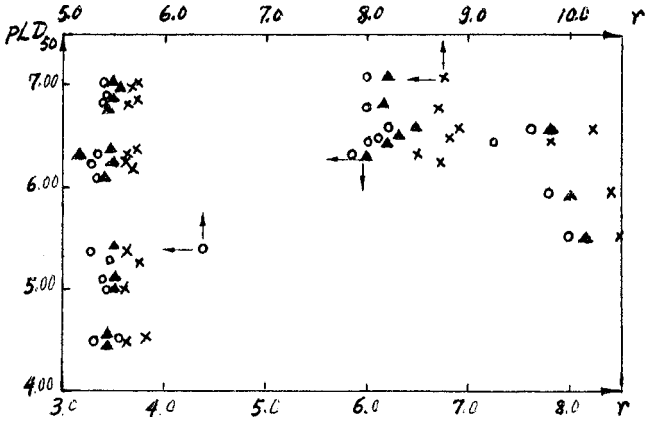

Figure 4. Correlation of the nearest distance *r* in conformations II (▲), III (○), and IV (×) with larvicidal activity pLD_{50} of SBH for halogen substituents connecting with benzene B.

dibenzoyl-1-*tert*-butylhydrazines in the study of structure−activity relationship.

It was well-known that the biological activity of RH 5849 mimics that of ecdysone. Most structure−activity relationship studies on ecdysone and its derivatives

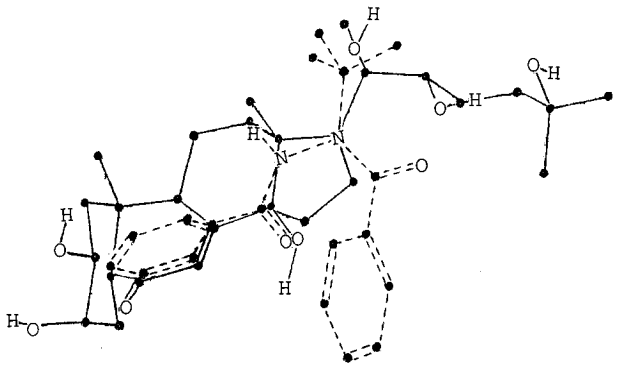

Figure 5. Overlap structure of dibenzoyl-1-*tert*-butylhydrazine (RH 5849) with 20-HE.

Table 4. Fitting Calculation of RH 5849 in Conformation I with 20-HE

trial	atom pairs	av deviation of atoms (Å)
1	O(1)−1′, N(1)−3′, N(2)−4′, C(15)−10′, O(2)−6′	0.711
2	C(7)−4′, O(1)−6′, N(1)−7′, N(2)−8′, C(15)−9′	0.336
3	C(11)−2′, C(12)−3′, C(7)−4′, C(13)−5′, O(1)−6′, N(1)−7′, N(2)−8′, C(15)−9′	0.477

suggested that there are three important regions, X, Y, and Z, of ecdysone involved in the binding interaction with receptor (Cohen et al., 1975; Thomson et al., 1970) (see Figure 1b). The A ring fits into a pocket at the receptor site, and important binding interactions take place on the β face (the X region). Particularly strong binding occurs at the Y region encompassing the 6-keto-7-ene and the 14-α-OH group in the α face. The Z region is the side chain with specific binding for the 22-OH group, but the interaction is less defined in the other part of the side chain.

The energy-minimized structure of 20-HE ($E = 61.03$ kcal/mol) was first obtained using the MMX-Minimize method. Comparing the three-dimensional structure of SBH in conformation I with that of 20-HE, we observe three common features: (i) both have a conjugated area [a benzene ring for SBH and a $\pi-$n conjugated bond for 20-HE (its B ring)]; (ii) both have a flexible alkyl group (*tert*-butyl for SBH and multicarbon flexible side chain for 20-HE); (iii) both have a strong electronegative charge center near a conjugated area (an oxygen atom at a carbonyl group near a benzene ring for SBH and an oxygen atom at the hydroxy group near the C=C−C=O bond for 20-HE). Therefore, our proposal is that atoms C(11), C(12), C(7), C(13), O(1), N(1), N(2), and C(15) of RH 5849 may mimic atoms 2′, 3′, 4′, 5′, 6′, 7′, 8′, and 9′ of 20-HE, respectively.

According to the above characteristics and the similarity between SBH in conformation I and 20-HE, certain atoms of the structure of RH 5849 in conformation I were superimposed on the minimized model of 20-HE using the "compare" function of the program, and a good overlap figure was obtained (see Figure 5). The relative average derivation of atoms in this fitting calculation including eight atom pairs was very low (see trial 3 in Table 4). This suggested the covered part might be believed as the common active site for SBH and 20-HE to activate the receptor.

Mohammed-Ali et al. (1995) and Chan et al. (1990) suggested O(1), N(1), N(2), C(15), and O(2) of RH 5849 may mimic atoms 1′, 3′, 4′, 10′, and 6′ of 20-HE and also carried out fitting calculations of the X-ray crystal structure of RH 5849 (A or B) and the ecdysone model without side chain; the relative average derivation of atoms in fits including five atom pairs was relatively high (0.745 Å) (Mohammed-Ali et al., 1995). We repeated the same fitting calculation of RH 5849 in conformation I with 20-HE, which was also very high (see trial 1 in Table 4). However, the fitting calculation based on our proposal including five atom pairs indeed gave very low relative average derivation of atoms (see trial 2 in Table 4), which at least implied that our proposal might be more reasonable.

LITERATURE CITED

Chan, T. H.; Ali, A.; Britten, J. F.; Thomas, A. W.; Strunz, G. M.; Salonius, A. The crystal structure of 1,2-dibenzoyl-1-*tert*-butylhydrazine, a nonsteroidal ecdysone agonist, and its effects on spruce budworm (*Choristoneura fumiferana*). *Can. J. Chem.* **1990**, *68*, 1178−1181.

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.* **1992**, *85*, 1099−1103.

Cohen, C. F.; Dutky, S. R.; Robbins, W. E.; Serban, N. N.; Shortino, T. J.; Svoboda, J. A.; Thompson, M. J. Synthesis and biological activity of 5α-hydroxy analogs of α-acdysone. *Lipids* **1975**, *10*, 615−620.

Darvas, B.; Polgar, L.; Tag El-Din, M. H.; Eroess, K.; Wing, K. D. Developmental disturbances in different insect orders caused by an ecdysteroid agonist, RH 5849. *J. Econ. Entomol.* **1992**, *85*, 2107−2112.

Kubo, I.; Klocke, J. A.; Asano, S. Effects of ingested phytoecdysteroids on the growth and development of two Lepidopterous larvae. *J. Insect Physiol.* **1983**, *29*, 307−316.

Mohammed-Ali, A. K.; Chan, T.-H.; Thomas, A. W.; Strunz, G. M.; Jewett, B. Structure-activity relationship study of synthetic hydrazines as ecdysone agonists in the control of spruce budworm (*Choristoneura fumiferana*). *Can. J. Chem.* **1995**, *73*, 550−557.

Oikawa, N.; Nakagawa, Y.; Nishimura, K.; Ueno, T.; Fujita, T. Quantitative structure-activity analysis of larvicidal 1-(substituted benzoyl)-2-benzoyl-1-*tert*-butylhydrazines against *Chilo suppressalis*. *Pestic. Sci.* **1994a**, *41*, 139−148.

Oikawa, N.; Nakagawa, Y.; Nishimura, K.; Ueno, T.; Fujita, T. Quantitative structure-activity studies of insect growth regulators: X. Substituent effects on larvicidal activity of 1-*tert*-butyl-1-(2-chlorobenzoyl)-2-(substituted benzoyl)hydrazines against *Chilo suppressalis* and design synthesis of potent derivatives. *Pestic. Biochem. Physiol.* **1994b**, *48*, 135−144.

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.* **1991**, *16*, 11−18.

Thomson, J. A.; Imary, F. P.; Horn, D. H. S. Improved calliphora bioassay for insect moulting hormones. *Aust. J. Exp. Biol. Med. Sci.* **1970**, *48*, 321−329.

Wing, K. D.; Slawecki, R. A.; Carlson, G. R. RH 5849, a nonsteroidal ecdysone agonist: effects on larval lepidoptera. *Science (Washington)* **1988a**, *241*, 467−469.

Wing, K. D.; Slawecki, R. A.; Carlson, G. R. RH 5849, a nonsteroidal ecdysone agonist: effects on larval lepidoptera. *Science (Washington)* **1988b**, *241*, 470−472.

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