

Quantitative Structure–Activity Relationships of Pine Weevil Antifeedants, a Multivariate Approach

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Antifeedant activity of mainly phenylpropanoic, cinnamic, and benzoic acids esters was tested on the pine weevil, *Hylobius abietis* (L.). Of 105 compounds screened for activity, 9 phenylpropanoates, 3 cinnamates, and 4 benzoates were found to be highly active antifeedants. To understand the structure–activity relationships of these compounds, a multivariate analysis study was performed. A number of molecular and substituent descriptors were calculated and correlated to results from two-choice feeding tests with *H. abietis*. Three local models were developed that had good internal predictive ability. External test sets showed moderate predictivity. In general, low polarity, small size, and high lipophilicity were characteristics for compounds having good antifeedant activity.

KEYWORDS: Conifer seedling; feeding deterrent; large pine weevil; *Hylobius abietis*; Curculionidae; benzoates; phenylpropanoates; cinnamates; phenylpropenoates; phenylacrylates; multivariate analysis; PLS; QSAR

INTRODUCTION

Weevils of the genus *Hylobius* are important pests of managed conifer forests in Europe, Asia, and North America (1). Several species injure healthy trees by larval feeding in the root collar region (2). In other species, the larvae develop in already dead or dying roots, and the economic damage is made by the adult weevils feeding on the stem bark of conifer seedlings (1). Severe damage is common in regions where replanting of harvested conifer forests is the prevalent forestry practice. In large parts of Europe, the pine weevil, *H. abietis*, is the most destructive pest of conifer regenerations, causing almost total mortality among seedlings if no countermeasures are taken (3, 4). Prophylactic treatment of seedlings with relatively persistent insecticides is therefore regularly carried out before planting (5). With the aim to abandon this use of insecticides, new methods for physical protection of seedlings are under develop-

ment in Sweden (5, 6). Efforts are also made to reduce damage levels by improvement of silvicultural measures (4, 7).

An alternative way of protecting conifer seedlings may be to treat the stem with a chemical substance being a feeding deterrent for *H. abietis* (8). Many substances with antifeedant effect have been identified for *H. abietis* in laboratory bioassays (8–14), and a few also for the closely related *H. pales* in North America (15). Much of the recent work with *H. abietis* has investigated categories of compounds that are present in pine weevil feces or in the bark of trees. Antifeedant compounds in *H. abietis* feces are of particular interest, because some of them may function as semiochemicals for this species (13). Feeding on root bark is to some extent avoided near oviposition sites (16), and this is apparently related to the fact that the female adds feces onto eggs laid in gnawed cavities in the bark (13). In that study, fractions from extracts of *H. abietis* feces were tested in feeding bioassays with *H. abietis*. From active fractions a number of compounds apparently originating from lignin were identified, including several benzoates. In a subsequent study (14), 55 commercially available or synthesized benzoic acid derivatives were bioassayed. The analogues with the highest antifeedant activity were esters of 2,4- and 3,5-dimethoxybenzoic acid. Two compounds with antifeedant effect on *H. abietis* have also been isolated from the bark of lodgepole pine (*Pinus*

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contorta) (8). These were ethyl 2,3-dibromophenyl propanoate and ethyl cinnamate, that is, both with nonsubstituted aromatic rings.

The aim of the present study was to find the structural criteria/chemical features required for an active pine weevil antifeedant and what structural changes can be made to improve the activity. For that reason, a number of analogues to the earlier isolated antifeedants (8, 13) were bought or synthesized and evaluated for antifeedant activity. The results from the previous study of the benzoates (14) were used as guidance in the selection of test compounds. We have mainly focused on esters of 3-phenylpropanoic, cinnamic, and benzoic acids. Complex relationships between structure and the activity of, among others, insect antifeedant and bird repellents have earlier successfully been studied with multivariate methods (17–20). In this study multivariate models based on a number of molecular and substituent descriptors were performed, correlating the antifeedant activity to chemical structure.

MATERIALS AND METHODS

General. ^1H NMR (400 MHz) and ^{13}C NMR (100.5 MHz) spectra were recorded on Varian Unity 400, Bruker 400, or Bruker 250 apparatus by using the solvent signals (CDCl_3 or CD_3OD) as internal standards. For TLC, silica gel plates with fluorescent indicator were used (Merck silica gel 60 F_{254} , 0.25 mm).

Chemicals. The following compounds were purchased from Sigma-Aldrich Co.: 2-hydroxy-4-methoxybenzoic acid (**B01**), 3,5-dihydroxybenzoic acid (**B02**), 4-hydroxy-3,5-dimethoxybenzoic acid (**B04**), 4-hydroxy-3-methoxybenzoic acid (**B06**), 4-hydroxybenzoic acid (**B07**), methyl 3-hydroxy-4-methoxybenzoate (**B14**), methyl 4-hydroxybenzoate (**B18**), 3-hydroxy-4-methoxybenzoic acid (**B23**), 3,4-methylenedioxybenzoic acid (**B25**), methyl 2-hydroxy-3-methoxybenzoate (**B27**), methyl 2,4-dimethoxybenzoate (**B32**), methyl 2,4,6-trimethoxybenzoate (**B33**), methyl 2,6-dimethoxybenzoate (**B35**), methyl 4-hydroxy-3-methoxybenzoate (**B37**), and methyl 3-(4-hydroxyphenyl)propanoate (**P25**).

The following compounds were purchased from Lancaster Synthesis Co.: 3,5-dimethoxybenzoic acid (**B03**), methyl 2,4-dihydroxybenzoate (**B08**), methyl 2-methoxybenzoate (**B10**), methyl 3-methoxybenzoate (**B15**), 2-hydroxy-3-methoxybenzoic acid (**B21**), 2-hydroxy-5-methoxybenzoic acid (**B22**), 3,4-dimethoxybenzoic acid (**B24**), 2-hydroxybenzoic acid (**B26**), methyl 2-hydroxy-5-methoxybenzoate (**B28**), methyl 3,5-dihydroxybenzoate (**B40**), methyl 3,5-dimethoxybenzoate (**B45**), methyl 4-methoxybenzoate (**B48**), and 3-(2-methoxyphenyl)propanoic acid (**P18**).

3-(2-Methylphenyl)propanoic acid (**P19**) was purchased from Matrix via Chemtronica, Stockholm, Sweden.

For syntheses of methyl 2,4-dihydroxy-3,6-dimethylbenzoate (**B09**), methyl 2,3-dimethoxybenzoate (**B11**), methyl 2,3,4-trimethoxybenzoate (**B12**), methyl 3,5-dinitrobenzoate (**B16**), methyl 3,5-dibromobenzoate (**B17**), isopropyl 2,4-dimethoxybenzoate (**B19**), 2,2,2-trifluoroethyl 3,5-dimethoxybenzoate (**B20**), methyl 2,5-dimethoxybenzoate (**B34**), methyl 3,4-dihydroxybenzoate (**B36**), methyl 3-chloro-4-methoxybenzoate (**B38**), methyl 3,4-methylenedioxybenzoate (**B39**), methyl 4-hydroxy-3,5-dimethoxybenzoate (**B43**), methyl 3,4,5-trimethoxybenzoate (**B44**), methyl 3,5-dimethylbenzoate (**B46**), *S*-ethyl 3,5-dimethoxybenzothioate (**B49**), *N*-ethyl 3,5-dimethoxybenzamide (**B50**), methyl 4-*n*-octylbenzoate (**B52**), dodecyl 3,4-dimethoxybenzoate (**B53**), 3-(*E*)-hexenyl 3,5-dimethoxybenzoate (**B54**), 2-methoxy-4-(2-propenyl)phenyl 3,5-dimethoxybenzoate (**B55**), and 3-(3,4-dimethoxyphenyl)propyl 3,5-dimethoxybenzoate (**B56**) see (14).

For syntheses of methyl 5-hydroxy-2-methoxybenzoate (**B13**), methyl 2-hydroxy-6-methoxybenzoate (**B29**), methyl 3-hydroxy-2-methoxybenzoate (**B30**), methyl 4-hydroxy-2-methoxybenzoate (**B31**), and methyl 3-hydroxy-5-methoxybenzoate (**B41**), see ref (11).

3,4,5-Triacetoxibenzoic acid (**B05**), 3,4,5-trimethoxybenzamide (**B51**), and methyl 3-(4-methoxyphenyl)propanoate (**P09**) were obtained

from previous work by H. Erdtman and T. Norin at the Department of Organic Chemistry, KTH, Stockholm.

The following esters were prepared by acid-catalyzed esterification of their corresponding commercial benzoic, propanoic, or *E*-propanoic acids: methyl 3,4,5-trihydroxybenzoate (**B42**), isopropyl 4-hydroxybenzoate (**B47**), methyl 2-methylpropanoate (**P01**), methyl 3-(3-methoxyphenyl)propanoate (**P02**), methyl 3-methylpropanoate (**P03**), methyl 3-(4-hydroxy-3-methoxyphenyl)propanoate (**P04**), methyl 3-(3-bromo-4-methoxyphenyl)propanoate (**P05**), methyl 3-(3,4-dichlorophenyl)propanoate (**P06**), methyl 3-(3,4,5-trimethoxyphenyl)propanoate (**P07**), methyl 3-(4-methylphenyl)propanoate (**P12**), methyl 3-(4-fluorophenyl)propanoate (**P14**), methyl 3-phenylpropanoate (**P20**), methyl 3-(2-methoxyphenyl)propanoate (**P21**), methyl 3-(2,4-dimethylphenyl)propanoate (**P23**), methyl 3-(4-isopropylphenyl)propanoate (**P26**), methyl 3-(4-chlorophenyl)propanoate (**P27**), methyl 3-(4-bromophenyl)propanoate (**P28**), ethyl 3-phenylpropanoate (**P29**), methyl 2-methyl-3-phenylpropanoate (**P31**), methyl 3-(2,3-dimethoxyphenyl)propanoate (**C01**), methyl 3-(2,4-dimethoxyphenyl)propanoate (**C02**), methyl 3-(3,4-dimethoxyphenyl)propanoate (**C03**), methyl 3-(3,5-dimethoxyphenyl)propanoate (**C04**), methyl 3-(4-methylphenyl)propanoate (**C05**), methyl 3-(4-isopropylphenyl)propanoate (**C06**), methyl 3-(4-trifluoromethylphenyl)propanoate (**C07**), methyl 3-(4-nitrophenyl)propanoate (**C09**), ethyl phenylpropanoate (**C10**), propyl phenylpropanoate (**C11**), isopropyl phenylpropanoate (**C12**), butyl phenylpropanoate (**C13**), and 2-butyl phenylpropanoate (**C14**).

Typical Esterification Procedure: Methyl (*E*)-3-(4-Methylphenyl)propanoate (C05**).** (*E*)-3-(4-Methylphenyl)propanoic acid (1.2 mmol) was dissolved in methanol (11 mL). Sulfuric acid (3 drops) was added. The mixture was refluxed for 4 h. The reaction mixture was concentrated using a rotary evaporator, diluted with water, and extracted with ethyl ether (30 mL). The ether phase was washed with $\text{Na}_2\text{CO}_3(\text{aq})$ (5 mL), dried over MgSO_4 , and evaporated to give white crystals (204 mg, 1.15 mmol); yield, 93%.

Methyl 3-(4-trifluoromethylphenyl)propanoate (**P13**), methyl 3-(2,3-dimethoxyphenyl)propanoate (**P22**), and methyl 3-(3,5-dimethoxyphenyl)propanoate (**P08**) were prepared by Pd/C-catalyzed hydrogenation of their corresponding propanoates according to the standard procedure (21).

Methyl 3-(4-aminophenyl)propanoate (**P15**) and methyl 3-(4-*N,N*-dimethylaminophenyl)propanoate (**P16**) were obtained by Pd/C-catalyzed hydrogenation of methyl 3-(4-nitrophenyl)propanoate and methyl 3-(4-*N,N*-dimethylaminophenyl)propanoate, respectively, according to the standard procedure (21).

Isopropyl 3-(4-methoxyphenyl)propanoate (**P17**) was obtained by reacting isopropyl 3-(4-hydroxyphenyl)propanoate with methyl iodide and potassium carbonate in acetone according to the standard procedure.

Methyl 3-(4-butoxyphenyl)propanoate (**P10**) was obtained by reacting methyl 3-(4-hydroxyphenyl)propanoate with potassium hydroxide and butyl iodide according to the standard procedure.

Methyl 3-(4-acetylphenyl)propanoate (**P11**) was obtained by refluxing methyl 3-(4-hydroxyphenyl)propanoate with an excess of acetic anhydride according to the standard procedure.

Methyl 3-(3,4-dimethoxyphenyl)propanoate (**P24**) was obtained by reacting methyl 3-(3,4-dihydroxyphenyl)propanoate with sodium hydride and methyl iodide in THF according to the standard procedure.

Methyl 3-(4-*N,N*-dimethylaminophenyl)propanoate (**C08**) was prepared from 4-(*N,N*-dimethylaminophenyl)propanoic acid by reaction with DCC and DMAP and methanol in CH_2Cl_2 (11).

Racemic mixtures (2*R*,3*S* and 2*S*,3*R*) of the esters methyl 2,3-dibromo-3-phenylpropanoate (**P34**) and ethyl 2,3-dibromo-3-phenylpropanoate (**P35**) were obtained by bromination of their corresponding *E*-phenylpropanoate, methyl phenylpropanoate and ethyl phenylpropanoate, respectively, according to the standard procedure (21). **P35** was also isolated from bark of *Pinus contorta*; see ref 8.

Ethyl 3-phenyl-3-hydroxypropanoate (**P32**) and ethyl 3-(2-bromophenyl)-3-hydroxypropanoate (**P33**) were obtained by adding ethyl bromoacetate to benzaldehyde and 2-bromobenzaldehyde, respectively, zinc dust, and copper acetate in THF according to the standard Reformatsky procedure (21).

(*S*)-Methyl 2-amino-3-phenylpropanoate (**P30**) was prepared by reacting L-phenylalanine with thionyl chloride followed by methanol according to the standard procedure (21).

All reactions were monitored by TLC. The spectroscopic data of the products were analyzed and compared with literature data.

Collection and Maintenance of Weevils. Both sexes of *H. abietis* were collected during spring migration at a sawmill in southern Sweden, where they landed in large numbers as a response to a massive emission of attractive conifer volatiles. After collection, the weevils were stored in darkness at 10 °C and provided with fresh Scots pine, *Pinus sylvestris* L., branches or stems with tender bark as food. These storage conditions interrupted the reproductive development, so that females did not begin to oviposit until about a week after they had been transferred to the experimental conditions, that is, a light regimen of L18 h /D6 h at 22 °C. This transfer was made about 10 days before the insects were used in the following bioassay.

Feeding Bioassay. The compounds were tested for their antifeedant effect on *H. abietis* by means of a two-choice laboratory bioassay (14), used in several previous studies (8, 11, 13, 14). For each test, 40 pine weevils (20 females + 20 males) were used. They were placed in separate Petri dishes provided with a Scots pine twig prepared with delimited treatment and control areas. These twigs were enveloped in aluminum foil, and two holes with a diameter of 5 mm and separated by 25 mm were punched in the foil with metal rings. After removal of the aluminum foil inside the rings, one of the two surfaces was treated with 100 μ L of a 50 mM methanol or methyl acetate solution of the compound tested, and the other surface was treated with the same amount of solvent alone. The following day, after the solvent had evaporated, the metal rings were removed and the test started. The proportion of available bark area that had been eaten by the test weevil on the treatment and control area of each test twig was recorded after 24 h. There was generally no significant difference in response between the sexes, and the data presented were therefore pooled.

The antifeedant effect measured for each compound is expressed by means of an antifeedant index (AFIa) based on feeding area (13, 14)

$$\text{AFIa} = 100 \times (C - T) / (C + T)$$

where *C* represents the mean area of control surfaces consumed and *T* the mean consumed area of treated surfaces. Positive values (up to a maximum of 100) reflect an antifeedant effect, whereas negative values (down to a minimum of -100) indicate a stimulant effect on feeding.

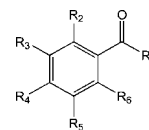
Multivariate Analysis. All molecular descriptors were calculated with Sybyl (22) and are described in Table 4. For the substituents on the aromatic ring (*R*₂–*R*₆) σ and π values were taken from the literature (23). Indicator variables were used to account for the substitution pattern. Score values (*t*_{1_subst}, *t*_{2_subst}, and *t*_{3_subst}) were also calculated for the different aromatic substituents (*R*₂–*R*₆) using principal component analysis (PCA) and used as descriptors. The PCA was based on σ_m , σ_p , π , molecular refractivity, and the five Verloop steric parameters (23). This analysis gave an *R* (2) = 0.75 and a *Q*² = 0.35 (*N* = 3).

A similar characterization was made of the ester substituent, that is, the whole substituent in position 1 on the ring [–COR₁, –(CH₂)₂COR₁, and –CH=CHCOR₁]. In the case of missing σ and π values they were calculated using the ACD/sigma program (24). Score values for the ester substituent (*t*_{1_ester}, *t*_{2_ester}, and *t*_{3_ester}) were obtained using σ_m , σ_p , π , and molecular refractivity, and a PCA was performed (*R*² = 0.96, *Q*² = 0.85, *N* = 3).

Simca-P+ (25) with autoscaling was used for the multivariate analysis studies. A PCA was first performed on the whole data set of 105 compounds (Tables 1–3) using 53 descriptors (Table 4). The data set was later divided into three groups corresponding to benzoic acid, 3-phenylpropanoic acid, and cinnamic acid derivatives. These three groups were used to derive local PLS models. In the class of benzoic acid derivatives, five compounds were removed (**B52**–**B56**), because they were structurally very different compared to the others.

The benzoic acid and 3-phenylpropanoic acid derivatives were divided into training and test sets on the basis of structural diversity using a full factorial design in three variables. In this design each variable is explored at two levels, low and high. The variables were derived from a PCA on the benzoic acid and the 3-phenylpropanoic

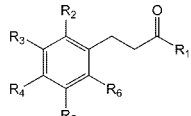
Table 1. Benzoic Acid Derivatives (When *R* Is Hydrogen, It Is Omitted from the Table)



subst no.	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	AFIa obsd	AFIa pred
<i>training set</i>								
B01	OH	OH		OMe			24	31
B02	OH		OH		OH		41	11
B03	OH		OMe		OMe		–4	29
B04	OH		OMe	OH	OMe		8	16
B05	OH		OAc	OAc	OAc		14	8
B06	OH		OMe	OH			48	26
B07	OH			OH			31	27
B08	OMe	OH		OH			46	42
B09	OMe	OH	Me	OH		Me	31	44
B10	OMe	OMe					80	64
B11	OMe	OMe	OMe				73	62
B12	OMe	OMe	OMe	OMe			54	60
B13	OMe	OMe			OH		–3	43
B14	OMe		OH	OMe			65	53
B15	OMe		OMe				89	61
B16	OMe		NO ₂		NO ₂		34	27
B17	OMe		Br		Br		50	61
B18	OMe			OH			34	50
B19	OiPr	OMe		OMe			96	98
B20	OCH ₂ CF ₃		OMe		OMe		86	82
<i>test set</i>								
B21	OH	OH	OMe				22	33
B22	OH	OH			OMe		17	24
B23	OH		OH	OMe			51	30
B24	OH		OMe	OMe			7	37
B25	OH			OCH ₂ O			14	35
B26	OMe	OH					21	56
B27	OMe	OH	OMe				95	54
B28	OMe	OH			OMe		74	46
B29	OMe	OH				OMe	74	56
B30	OMe	OMe	OH				75	56
B31	OMe	OMe		OH			35	49
B32	OMe	OMe		OMe			99	60
B33	OMe	OMe		OMe		OMe	55	59
B34	OMe	OMe			OMe		89	55
B35	OMe	OMe				OMe	51	63
B36	OMe		OH	OH			–7	42
B37	OMe		OMe	OH			53	47
B38	OMe		Cl	OMe			36	61
B39	OMe			OCH ₂ O			57	59
B40	OMe		OH		OH		23	34
B41	OMe		OH		OMe		54	45
B42	OMe		OH	OH	OH		21	21
B43	OMe		OMe	OH	OMe		10	39
B44	OMe		OMe	OMe	OMe		32	50
B45	OMe		OMe		OMe		95	52
B46	OMe		Me		Me		61	63
B47	OiPr			OH			63	86
B48	OMe			OMe			54	61
B49	SEt		OMe		OMe		74	71
B50	NHEt		OMe		OMe		86	59
B51	NH ₂		OMe	OMe	OMe		–2	16
B52	OMe			n-C ₈ H ₁₇			35	98
B53	O-C ₁₂ H ₂₅		OMe	OMe			23	251
B54	a		OMe		OMe		62	131
B55	b		OMe		OMe		17	207
B56	c		OMe		OMe		41	221

^a (*E*)-3-Hexenyl-. ^b 2-Methoxy-(4-prop-2-enyl)phenyl-. ^c 3-(3,4-Dimethoxyphenyl)propyl-.

acid derivatives separately (Table 5) using the 53 descriptors (Table 4). Two substances were selected at each level, and four centerpoints were used. For the 3-phenylpropanoic acid derivatives the low–high–high variable level contained only one compound, which was included

Table 2. Propanoic Acid Derivatives (When R Is Hydrogen, It Is Omitted from the Table)


Subst no	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	AFIa observed	AFIa predicted
<i>Training set</i>								
P01	OMe	Me					91	98
P02	OMe		OMe				95	74
P03	OMe		Me				90	94
P04	OMe		OMe	OH			54	35
P05	OMe		Br	OMe			68	72
P06	OMe		Cl	Cl			98	99
P07	OMe		OMe	OMe	OMe		26	40
P08	OMe		OMe		OMe		89	58
P09	OMe			OMe			96	75
P10	OMe			OBu			70	84
P11	OMe			OAc			14	48
P12	OMe			Me			99	98
P13	OMe			CF ₃			57	81
P14	OMe			F			98	90
P15	OMe			NH ₂			29	36
P16	OMe			NMe ₂			84	82
P17	O <i>i</i> Pr			OMe			83	77
<i>Test set</i>								
P18	OH	OMe					12	63
P19	OH	Me					47	81
P20	OMe						44	95
P21	OMe	OMe					97	83
P22	OMe	OMe	OMe				86	65
P23	OMe	Me		Me			100	103
P24	OMe		OMe	OMe			35	59
P25	OMe			OH			21	51
P26	OMe			<i>i</i> Pr			94	104
P27	OMe			Cl			100	100
P28	OMe			Br			97	96
P29	OEt						73	99
P30 ^a							18	50
P31							79	97
P32 ^b							36	64
P33 ^c							59	64
P34 ^d							50	88
P35							45 ^c /75 ^d	91

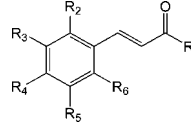
^a Enantiomerically pure, synthesized from L-phenylalanine. ^b *R/S* mixtures. ^c *R,S* mixture. ^d Isolated from *Pinus contorta*.

in the training set. The five racemic compounds (**P31–P35**) were placed in the test set. Furthermore, no compounds from the high–low–high level were chosen, because it contained only racemic compounds.

In the derivation of the final PLS models, variables with little importance, as judged from the VIP plot and the coefficient plot, were excluded.

RESULTS AND DISCUSSION

Antifeedant activity was measured for 35 3-phenylpropanoic, 14 cinnamic, and 56 benzoic acid derivatives in the bioassay with *H. abietis*. Nine phenylpropanoates, three cinnamates, and four benzoates were very active antifeedants, having an AFIa of 95–100 (Tables 1–4). None of the tested phenylpropanoids stimulated feeding (i.e., negative AFIa values), as was the case for some of the benzoic acid derivatives (Table 1) (14). All acids tested (12 benzoic and 2 phenylpropanoic acids) had low activities, which is in agreement with previous findings by Ericsson (17). Also, esters with hydroxy or other polar substituents

Table 3. Cinnamic Acid Derivatives (When R Is Hydrogen, It Is Omitted from the Table)


subst no.	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	AFIa obsd	AFIa pred
C01	OMe	OMe	OMe				99	103
C02	OMe	OMe		OMe			96	93
C03	OMe		OMe	OMe			36	33
C04	OMe		OMe		OMe		72	46
C05	OMe			Me			76	85
C06	OMe			<i>i</i> Pr			92	82
C07	OMe			CF ₃			62	65
C08	OMe			NMe ₂			49	58
C09	OMe			NO ₂			32	40
C10	OEt						83	83
C11	OPr						85	72
C12	O <i>i</i> Pr						96	79
C13	OBu						38	59
C14	O-2-Bu						48	67

uents had low activities (AFIa < 50). In another study of insect feeding deterrents (26), it was also found that nonpolar substituents on low molecular weight aromatic compounds increased the activity. It has also been shown that other lipophilic compounds including monoterpenoids (10, 15, 27, 28), nonanoic acid (12), allylanisole (10), dihydropinidine (29), and a number of substituted cinnamic aldehydes, esters, and benzaldehydes (17) are potent pine weevil antifeedants. Among the more lipophilic substances in our data set, the activity varied substantially. For example, compound **P07**, methyl 3-(3,4,5-trimethoxyphenyl)propanoate, had low activity (AFIa = 26), whereas pine weevils were totally deterred by twigs treated with **P23**, methyl 3-(2,4-dimethylphenyl)propanoate (AFIa = 100). Similar complex structure–activity relationships were also observed by Ericsson (17).

Besides the apparent observations mentioned above, the structure–activity relationships were not easy to interpret. Thus, a multivariate analysis of the data was performed. In this study each compound was described with 52 variables describing general molecular properties such as lipophilicity, polarity, electronic properties, size, and substitution pattern (Table 4), and these were correlated to antifeedant activity (AFIa) (Tables 1–3).

A PCA was first performed in Simca using the whole data set of 105 molecules and 53 molecular descriptors (including AFIa). In this analysis three components were extracted that explained 49% of the variance in the data set. Because the score plot (Figure 1) showed a clear grouping in the first component between the benzoic acid derivatives, on the one hand, and the phenylpropanoic/cinnamic acid derivatives, on the other, we created local models based on the three structural classes separately. Efforts to derive PLS models including all compounds were unsuccessful.

After variable reduction, final PLS models were derived that included 11, 8, and 11 descriptors for the benzoic acid, the 3-phenylpropanoic acid, and the cinnamic acid derivatives, respectively (Figure 2A,C,E). The observed versus predicted values (Tables 13) for the three different models are shown in Figure 2B,D,F. The rmsEP and R^2_{pred} values for the test sets are shown in Table 5. The external predictivity was moderate with an R^2_{pred} of 0.38 and 0.34 for the benzoic acid derivatives and 3-phenylpropanoic acid derivatives, respectively. PLS

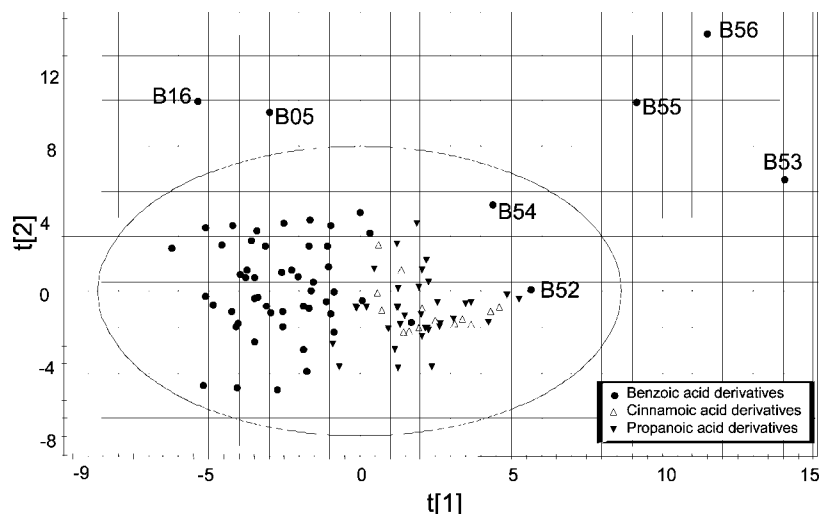


Figure 1. Score plot of the first and second principal components for the PCA of all compounds. Local PLS models were based on the separation observed between the benzoic acid derivatives, on the one hand, and the 3-phenylpropanoic/cinnamic acid derivatives, on the other, captured in the first principal component. The large lipophilic benzoic acid derivatives (B52–B56) correspond to the five solid circles to the right.

Table 4. Description of the Variables Used in the Modeling

	Substituent Descriptors for R ₂ –R ₆
$\sigma_{2,3,4,5, \text{ or } 6}$	sigma meta and sigma para (23) for the substituents R ₂ –R ₆ , which describe electronic properties of the substituent in the meta or para position, respectively
$\pi_{2,3,4,5, \text{ or } 6}$	pi value (23) for the substituents R ₂ –R ₆ , which describe lipophilic properties of the substituent
$I_{2,3,4,5, \text{ or } 6}$	indicator variable for the substituents R ₂ –R ₆ (0 = hydrogen, 1 = any other substituent)
L, B1, B2, B3, B4	Verloop's steric parameters for the substituents R ₂ –R ₆ (23)
$t_{1_subst}, t_{2_subst}, t_{3_subst}$	principal component values from a PCA of the different substituents R ₂ –R ₆ ; t_1 describes size and lipophilicity, t_2 describes electronic properties, and t_3 describes electronic properties together with size
	Substituent Descriptors for the Ester Substituent (–COR ₁ , –(CH ₂) ₂ COR ₁ , and –CH=CHCOR ₁)
MR ₁	molecular refractivity for the ester substituent calculated with the ACD/sigma program (24) describes the steric or “bulk” properties of this substituent
MW ₁	molecular weight for the ester substituent calculated with the ACD/sigma program (24)
π_1	pi value for the ester substituent calculated with the ACD/sigma program (24), which describes lipophilic properties of this substituent
$\sigma(\text{Ind})$	inductive sigma value calculated with the ACD/sigma program (24), which describes electronic properties of the ester substituent
$\sigma(\text{Res})$	resonance sigma value calculated with the ACD/sigma program (24), which describes electronic properties of the ester substituent
$t_{1_ester}, t_{2_ester}, t_{3_ester}$	principal component values from a PCA of the different ester substituents; t_1 describes electronic properties, t_2 describes lipophilic properties, and t_3 describes the difference between $s(\text{Res})$ and $s(\text{Ind})$
	Molecular Descriptors, Calculated with Sybyl Version 6.9 (22)
CLOGP	calculated log partition coefficient octanol–water
CMR	calculated molecular refractivity: $MR = (MW/d) \times [(\eta^2 - 1)/(\eta^2 + 2)]$, where MW is molecular weight, d is density, and η is refractive index; CMR describes the steric or “bulk” properties of the whole molecule
RingCount	number of rings
AtomCount	number of atoms
BondCount	number of bonds
RotBonds	number of rotatable bonds
HB_ACC	number of hydrogen bond acceptors
HB_DON	number of hydrogen bond donors
HB_ALL	sum of the number of hydrogen bond donors and acceptors: $\text{HB_ALL} = \text{HB_ACC} + \text{HB_DON}$
MW _{total}	molecular weight
AREA	molecular surface area
VOLUME	molecular volume
PV	polar volume, i.e., the volume of all nitrogen, oxygen, and sulfur atoms as well as hydrogens covalently bonded to these atoms
PSA	polar surface area, i.e., the surface area of all nitrogen, oxygen, and sulfur atoms as well as hydrogens covalently bonded to these atoms
	Descriptor for Biological Activity
AFIa	antifeedant index

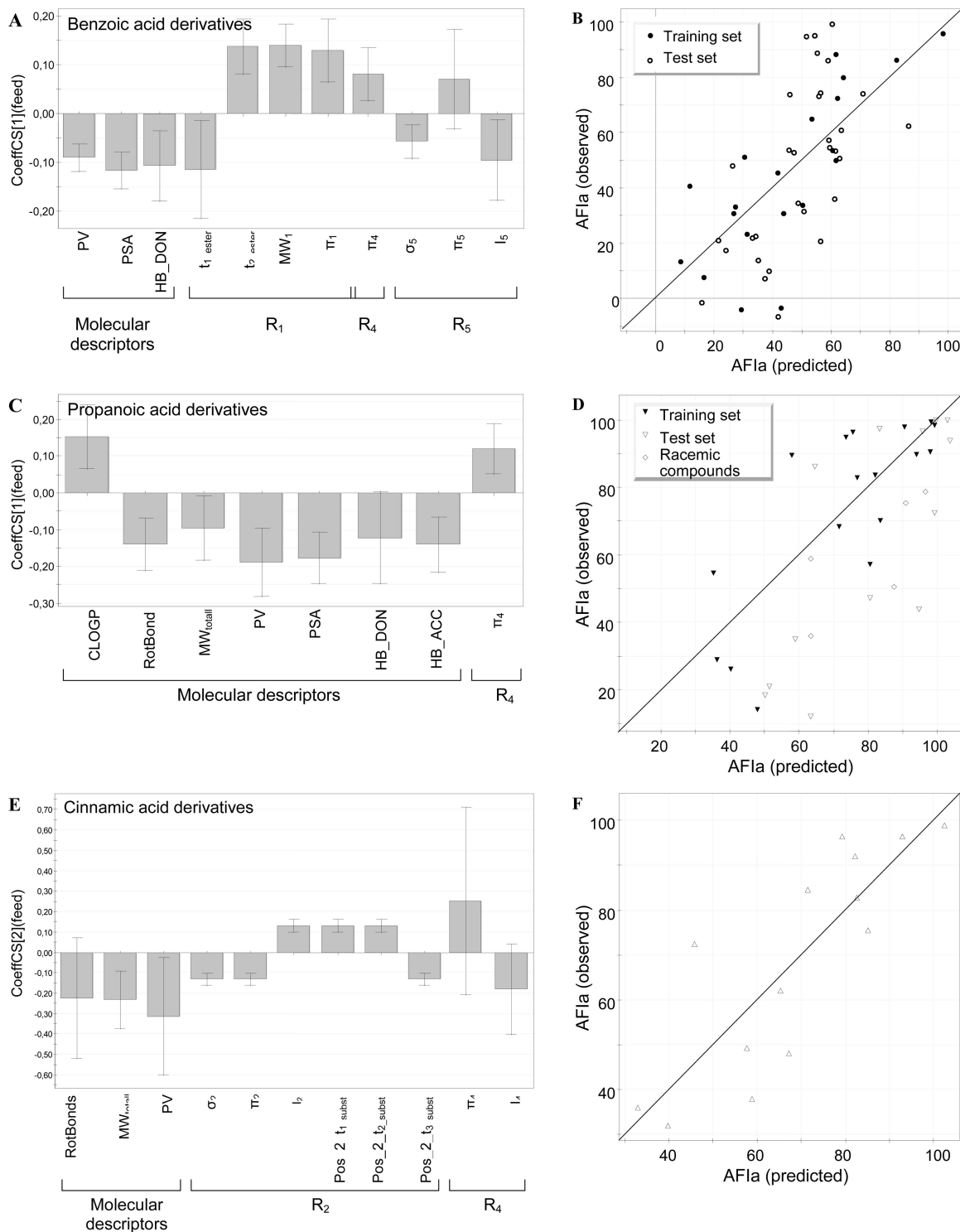


Figure 2. (A) Coefficient plot for PLS model for the benzoic acid derivatives. Positive columns mean that the variables are positively correlated to antifeedant activity. Negative columns are variables in which high values diminish the antifeedant activity. (B) Observed versus predicted plot for the training set and external test set for the benzoic acid derivatives. Similar plots are shown for the 3-phenylpropanoic acid derivatives (C, D) and the cinnamates (E, F).

models with scrambled y values produced Q^2 values that were mostly negative or very low.

In the PCA plot in **Figure 1**, the five benzoic acid derivatives (B52–B56) appear as outliers in the first principal component and were not considered in the modeling. These compounds contain long alkyl chains (**Table 1**) and are therefore larger and also have a higher lipophilicity as compared to the other benzoic acid derivatives. All other compounds were included in the modeling.

In general, for all three PLS models, an overall low polarity and size and a high lipophilicity are characteristic for a compound having good antifeedant activity. In the three PLS models this trend is captured by different combinations of descriptors such as polar volume, polar surface area, hydrogen bond donors and acceptors, molecular weight, and calculated octanol–water partition coefficient (CLOGP). For example, MW_{total} is negatively correlated to AFIa (**Figure 2C,E**). The molecular descriptors PV and PSA, which partly describe size,

Table 5. Results from Multivariate Modeling

	benzoic acid derivatives	propanoic acid derivatives	cinnamic acid derivatives
PCA			
R^2 ^a	0.52	0.50	0.72
Q^2 ^b	0.23	0.04	0.36
N ^c	3	3	3
PLS			
R^2 ^a	0.61	0.63	0.70
Q^2 ^b	0.55	0.56	0.53
N ^c	1	1	2
$n_{\text{training set}}$ ^d	20	17	14
$n_{\text{test set}}$ ^d	31	13 (18)	
R^2_{pred} ^e	0.38	0.34 (0.28) ^g	
RMSEP ^f	24	28 (27) ^g	

^a Explained variation or goodness of fit. ^b Predicted variation or goodness of prediction calculated from the training set. ^c Number of components. ^d Number of compounds in the training set and test set. ^e $R^2_{\text{pred}} = 1 - \text{PRESS}/\text{SD}$, where PRESS stands for predictive sum of squares and SD for sum of squared differences. ^f Root-mean-square error of prediction for the test set. ^g rmsEP and R^2_{pred} are given for the test set without the racemic compounds and in parentheses for the whole test set.

are also negatively correlated to AFIA. These two descriptors also describing polarity together with the hydrogen-bonding descriptors (HB_DON and HB_ACC) capture the trend that a high-polarity diminishes AFIA (**Figure 2A,C,E**) and a high CLOGP value increases the AFIA (**Figure 2C**). The positive effect of a high lipophilicity is also captured by the $t_{2\text{ester}}$, π_1 , π_4 , and π_5 descriptors in the different substituent positions (R_1 , R_4 , and R_5). A para substituent with a high π value (high lipophilicity) gives a high antifeedant activity in all three models (**Figure 2A,C,E**). In the benzoic acid derivatives this can be exemplified by comparing compounds that differ only in the para position, for example, in order of increasing activity **B43** ($R_4 = \text{OH}$), **B44** ($R_4 = \text{OMe}$), and **B45** ($R_4 = \text{H}$) with AFIA values of 10, 32, and 95, respectively. In the propanoic acid derivatives the para substituent is the most varied in the whole data set. Seven of the 12 different para substituents tested gave an antifeedant activity of >83 , and the 3 compounds (**P11**, **P15**, and **P25**) with the lowest AFIA also have very low π_4 values (-1.23 to -0.64).

For the benzoic acid derivatives the esters are generally better antifeedants than the carboxylic acids (**Table 1**). This general trend has been observed previously (17). This observation is also supported in this study by the model descriptors MW_1 , π_1 and $t_{2\text{ester}}$ in **Figure 2A**. However, there appears to be an optimum concerning the size or lipophilicity of the alcohol moiety, because compounds (**B53–B56**) with very large alkyl groups such as alcohol moieties do not exert high antifeedant activity. In the analysis of the benzoic acid derivatives an R_5 substituent corresponding to a methoxy group or a hydrogen atom seems to be beneficial for good antifeedant activity.

All five racemic propanoic acid derivatives (**P31–P35**) in the test set were overpredicted (**Figure 2D**). Thus, when the five racemic compounds in the test set were excluded, R^2_{pred} was improved (**Table 5**). In a previous study (8) ethyl 2,3-dibromopropanoate (**P35**) was isolated from *P. contorta*. The isolated compound had an AFIA of 75, whereas the synthetic *RS/SR* racemate obtained from the *E*-cinnamate had AFIA of 45. Unfortunately, the optical rotation was not determined for the isolated sample, but one explanation for these results might be that all four stereoisomers are not equally active and that an excess of the more active stereoisomer(s) is biosynthesized in the pines.

In the series of cinnamates, consisting of only 14 compounds, we could not obtain any models when the data set was divided into training and test sets. The PLS models were therefore derived using all of the cinnamates. The obtained PLS model showed that nonsubstituted cinnamates (**C10–C14**), with few rotatable bonds, had a higher antifeedant activity. The two compounds with a methoxy substituent (**C01** and **C02**) in the ortho position are among the most active, which is also reflected by the coefficients for all six R_2 descriptors.

Due to the nature of the PLS model, predicted AFIA values >100 are possible but are, in reality, impossible. Predicted AFIA values slightly larger than 100 as in the case for **P23**, **P26**, and **C01** should be interpreted as very promising compounds seen in relation to the root-mean-square error of prediction (rmsEP) for the model (**Table 5**) as for all quantitative predictions. Very unrealistic predictions as for **B53–B56** can be identified, as these compounds are already considered to be very different from the training set of the model.

The potential of using antifeedants to protect forest regeneration against pine damage has previously been demonstrated in field tests with methyl 3,5-dimethoxybenzoate (**B45**) (30) and ethyl 2,3-dibromo-3-phenylpropanoate (**P35**).

In this study, several highly active antifeedants have been identified among the phenylpropanoates and cinnamates. The multivariate models have given a better understanding not only of which structural properties are important for high antifeedant activity but also of how to optimize the activity within the three compound classes.

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LITERATURE CITED

- (1) Day, K. R.; Nordlander, G.; Kenis, M.; Halldórsson, G. General biology and life cycles of bark weevils. In *Bark and Wood Boring Insects in Living Trees in Europe*; Lieutier, F., Day, K. R., Battisti, A., Grégoire, J.-C., Evans, H. F., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2004; pp 331–349.
- (2) Cerezke, H. F. Warren rootcollar weevil, *Hylobius warreni* Wood (Coleoptera: Curculionidae), in Canada: ecology, behaviour, damage relationships, and management. *Can. Entomol.* **1994**, *126*, 1383–1442.
- (3) Örlander, G.; Nilsson, U. Effect of reforestation methods on pine weevil (*Hylobius abietis*) damage and seedling survival. *Scand. J. For. Res.* **1999**, *14*, 341–354.
- (4) Petersson, M.; Örlander, G. Effectiveness of combinations of shelterwood, scarification, and feeding barriers to reduce pine weevil damage. *Can. J. For. Res.* **2003**, *33*, 64–73.
- (5) Långström, B.; Day, K. R. Damage, control and management of weevil pests, especially *Hylobius abietis*. In *Bark and Wood Boring Insects in Living Trees in Europe*; Lieutier, F., Day, K. R., Battisti, A., Grégoire, J.-C., Evans, H. F., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2004; pp 415–444.
- (6) Petersson, M.; Örlander, G.; Nilsson, U. Feeding barriers to reduce damage by pine weevil (*Hylobius abietis*). *Scand. J. For. Res.* **2004**, *19*, 48–59.
- (7) Wallertz, K.; Örlander, G.; Luoranen, J. Damage by pine weevil *Hylobius abietis* to conifer seedlings after shelterwood removal. *Scand. J. For. Res.* **2005**, *20*, 412–420.
- (8) Bratt, K.; Sunnerheim, K.; Nordenhem, H.; Nordlander, G.; Långström, B. Pine weevil (*Hylobius abietis*) antifeedants from lodgepole pine (*Pinus contorta*). *J. Chem. Ecol.* **2001**, *27*, 2253–2262.

- (9) Lindgren, B. S.; Nordlander, G.; Birgersson, G. Feeding deterrence of verbenone to the pine weevil, *Hylobius abietis* (L.) (Col., Curculionidae). *J. Appl. Entomol.* **1996**, *120*, 397–403.
- (10) Klepzig, K. D.; Schlyter, F. Laboratory evaluation of plant-derived antifeedants against the pine weevil *Hylobius abietis* (Coleoptera: Curculionidae). *J. Econ. Entomol.* **1999**, *92*, 644–650.
- (11) Legrand, S.; Nordlander, G.; Nordenhem, H.; Borg-Karlson, A.-K.; Unelius, C. R. Hydroxy-methoxybenzoic methyl esters: synthesis and antifeedant activity on the pine weevil, *Hylobius abietis*. *Z. Naturforsch.* **2004**, *59B*, 829–835.
- (12) Månsson, P. E.; Eriksson, C.; Sjödin, K. Antifeedants against *Hylobius abietis* pine weevils: an active compound in extract of bark of *Tilia cordata* linden. *J. Chem. Ecol.* **2005**, *31*, 989–1001.
- (13) Borg-Karlson, A. K.; Nordlander, G.; Mudalige, A.; Nordenhem, H.; Unelius, C. R. Antifeedants in the feces of the pine weevil *Hylobius abietis*: identification and biological activity. *J. Chem. Ecol.* **2006**, *32*, 943–957.
- (14) Unelius, C. R.; Nordlander, G.; Nordenhem, H.; Hellqvist, C.; Legrand, S.; Borg-Karlson, A. K. Structure–activity relationships of benzoic acid derivatives as antifeedants for the pine weevil, *Hylobius abietis*. *J. Chem. Ecol.* **2006**, *32*, 2191–2203.
- (15) Salom, S. M.; Carlson, J. A.; Ang, B. N.; Grossman, D. M.; Day, E. R. Laboratory evaluation of biologically based compounds as antifeedants for the pales weevil, *Hylobius pales* (Herbst) (Coleoptera: Curculionidae). *J. Entomol. Sci.* **1994**, *29*, 407–419.
- (16) Bylund, H.; Nordlander, G.; Nordenhem, H. Feeding and oviposition rates in the pine weevil *Hylobius abietis* (Coleoptera: Curculionidae). *Bull. Entomol. Res.* **2004**, *94*, 307–317.
- (17) Eriksson, C. Isolation, synthesis and structure–activity relationships of antifeedants against the pine weevil, *Hylobius abietis*. Doctoral thesis in organic chemistry, KTH, Stockholm, Sweden, 2006.
- (18) Cao, S.; Wei, N.; Zhao, C.; Li, L.; Huang, Q.; Qian, X. Syntheses, antifeedant activity, and QSAR analysis of new oxa(thia)diazoyl 3(2*H*)-pyridazinones. *J. Agric. Food Chem.* **2005**, *53*, 3120–3125.
- (19) Morimoto, M.; Tanimoto, K.; Nakano, S.; Ozaki, T.; Nakano, A.; Komai, K. Insect antifeedant activity of flavones and chromones against *Spodoptera litura*. *J. Agric. Food Chem.* **2003**, *51*, 389–393.
- (20) Watkins, R. W.; Lumley, J. A.; Gill, E. L.; Bishop, J. D.; Langton, S. D.; MacNicol, A. D.; Price, N. R.; Drew, M. G. B. Quantitative structure–activity relationships (QSAR) of cinnamic acid bird repellents. *J. Chem. Ecol.* **1999**, *25*, 2825–2845.
- (21) Vogel. *Textbook of Practical Organic Chemistry*, 5th ed.; Prentice Hall: Engelwood Cliffs, NJ, 1989.
- (22) Sybyl 6.9, Tripos Inc., St. Louis, MO.
- (23) Seydel, J. K.; Schaper, K.-J. *Chemische Struktur und Biologische Aktivität von Wirkstoffen*; Verlag Chemie: Weinheim, Germany, 1979; pp 266–288.
- (24) ACD/ChemSketch& ACD/Sigma, version 4.55, Advanced Chemistry Development, Inc., Toronto, ON, Canada, 2000; www.acdlabs.com.
- (25) Simca-P+, version 10.0, Umetrics, Umeå, Sweden, 2002; www.umetrics.se.
- (26) Harmatha, J.; Nawrot, J. Insect feeding deterrent activity of lignans and related phenylpropanoids with a methylenedioxyphenyl (piperonyl) structure moiety. *Entomol. Exp. Appl.* **2002**, *104*, 51–60.
- (27) Salom, S. M.; Gray, J. A.; Alford, A. R.; Muleski, M.; Fettig, C. J.; Woods, S. A. Evaluation of natural products as antifeedants for the pales weevil (Coleoptera: Curculionidae) and as fungitoxins for *Leptographium procerum*. *J. Entomol. Sci.* **1996**, *31*, 453–465.
- (28) Schlyter, F.; Smitt, O.; Sjödin, K.; Högborg, H.-E.; Löfqvist, J. Carvone and less volatile analogues as repellent and deterrent antifeedants against the pine weevil, *Hylobius abietis*. *J. Appl. Entomol.* **2004**, *128*, 610–619.
- (29) Eriksson, C.; Sjödin, K.; Schlyter, F.; Högborg, H.-E. Synthesis of (+)- and (–)-dihydropinidine by diastereoselective dimethylzinc promoted allylation of 2-methyltetrahydropyridine-*N*-oxide with an allylboronic ester. *Tetrahedron Asymmetry* **2006**, *17*, 1074–1080.
- (30) Nordlander, G.; Nordenhem, H.; Borg-Karlson, A.-K.; Unelius, R. Use for conifer sapling protection. Swedish and PCT Patent WO 0056152 A1, **2000**.

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