J Chem Ecol (2008) 34:339–352
DOI 10.1007/s10886-008-9435-1

Structure–Activity Relationships of Phenylpropanoids as Antifeedants for the Pine Weevil *Hylobius abietis*

B. Bohman · G. Nordlander · H. Nordenhem · K. Sunnerheim · A.-K. Borg-Karlson · C. R. Unelius

Received: 23 July 2007 / Revised: 19 December 2007 / Accepted: 16 January 2008 / Published online: 29 February 2008

Abstract Ethyl cinnamate has been isolated from the bark of *Pinus contorta* in the search for antifeedants for the pine weevil, *Hylobius abietis*. Based on this lead compound, a number of structurally related compounds were synthesized and tested. The usability of the Topliss scheme, a flow diagram previously used in numerous structure–activity relationship (SAR) studies, was evaluated in an attempt to find the most potent antifeedants. The scheme was initially followed stepwise; subsequently, all compounds found in the scheme were compared. In total, 51 phenylpropanoids were tested and analyzed for SARs by using arguments from the field of medicinal chemistry (rational drug design). Individual Hansch parameters based on hydrophobicity, steric, and electronic properties were examined. The effects of position and numbers of substituents on the aromatic ring, the effects of conjugation in the molecules, and the effects of the properties of the parent alcohol part of the esters were also evaluated. It proved difficult to find strong SARs derived from single physicochemical descriptors, but our study led to numerous new, potent, phenylpropanoid antifeedants for the pine weevil. Among the most potent were methyl 3-phenylpropanoates monosubstituted with chloro, fluoro, or methyl groups and the 3,4-dichlorinated methyl 3-phenylpropanoate.

Keywords Antifeedant · Pine weevil · *Hylobius abietis* · Structure–activity · SAR · Phenylpropanoid · Phenylpropanoate · Phenylacrylate

B. Bohman · C. R. Unelius (✉)
School of Pure and Applied Natural Sciences,
University of Kalmar,
SE-391 82 Kalmar, Sweden
e-mail: rikard.unelius@hik.se

G. Nordlander · H. Nordenhem
Department of Ecology,
Swedish University of Agricultural Sciences,
P.O. Box 7044, SE-750 07 Uppsala, Sweden

K. Sunnerheim
Department of Natural Sciences, Mid Sweden University,
SE-851 70 Sundsvall, Sweden

A.-K. Borg-Karlson
KTH Chemistry, Organic Chemistry,
Ecological Chemistry Group, Royal Institute of Technology,
SE-100 44 Stockholm, Sweden

Introduction

The pine weevil *Hylobius abietis* (L.) is a severe pest in areas of Europe where clear-cutting of conifer forests with subsequent replanting is practiced (Långström and Day 2004) because newly planted seedlings are frequently killed by the feeding of adult weevils on the stem bark (Day et al. 2004). There is a strong demand in Sweden and other European countries for new methods that prevent this pest from damaging forest plantations without the use of pesticides (Långström and Day 2004). Pine weevils walking on the ground locate the seedlings by responding to both olfactory and visual stimuli (Björklund et al. 2005).

Previously, it has been shown that lodgepole pine, *Pinus contorta* Douglas ex Loudon, is not as severely attacked as Scots pine, *P. sylvestris* L. (Bratt et al. 2001). This observation led to the hypothesis that the bark of *P. contorta* contains secondary metabolites that act as natural antifeedants against *H. abietis*. This was confirmed in laboratory bioassays where bark extracts from *P. contorta* deterred feeding more than bark extracts from *P. sylvestris*. Bioassay-guided fractionation of *P. contorta* bark led to the

isolation and identification of two compounds, ethyl cinnamate and ethyl 2,3-dibromo-3-phenylpropanoate, which possessed antifeedant activity (Bratt et al. 2001). Ethyl cinnamate and structurally related esters are more potent antifeedants than the corresponding carboxylic acids (Sunnerheim et al. 2007). The results of an investigation of benzoate derivatives as antifeedants for *H. abietis* indicated that even small changes in the structure of a derivative might induce dramatic effects on the antifeedant activity (Unelius et al. 2006). Therefore, it is important to understand the relation between the substitution pattern of the aromatic ring and also the antifeedant effect in phenylpropanoids.

When looking for new bioactive compounds, it might not be feasible to test a large number of structures to find the most potent one. Under these circumstances, it is beneficial to test compounds for activity as they are synthesized and to use the results to select the next analog to be tested. These ideas are applied in the Topliss approach (Topliss 1972), which we used as a tool in search for the phenylpropanoid with the highest antifeedant effect. A Topliss scheme is a flow diagram designed such that the optimal substituent can be found expediently (Topliss 1977), and it gives recommendations on how to proceed after each compound has been tested. The substituents in the Topliss scheme have been chosen based on physico-chemical properties in combination with ease of synthesis. The test approach drawn up by Topliss is a modified and simplified quantitative SAR (QSAR) method developed from the Hansch method for structure–activity correlations. In the Hansch method, mathematical functions are used to correlate biological activity to chemical structure. For instance, the biological activities of a number of substituted aromatic compounds on bacteria, insects, and mammals have been examined and correlated to the properties of the substituents (Hansch and Fujita 1963). Depending on the structural configurations of the receptor active sites, different factors may affect efficiency. In this study, the relationships between individual Hansch parameters and potency of antifeedants against the pine weevil are presented.

To further explore the effects of structural changes in the aromatic rings of compounds such as phenylpropanoids, the number of substituents and the substitution pattern on the ring can be examined and evaluated in relation to activity, i.e., antifeedant efficacy. Certain positions are expected to be beneficial for a strong interaction to a target receptor. In addition to monosubstituted analogs, compounds with two or three substituents can also be tested and evaluated. As biological activity is potentially dependent upon the electronic properties of the entire molecule, it is important to investigate whether acrylates or propanoates are the superior antifeedants. The choice of parent alcohol for the ester is also likely to be important (Unelius et al. 2006).

The overall goal of the present study was to find new effective antifeedants related to ethyl cinnamate that could be used for conifer seedling protection. Throughout this process, we wanted to evaluate the Topliss approach for finding more potent antifeedants by using a low number of syntheses and biological tests. Individual Hansch parameters were also evaluated, as were the number, pattern, and properties of substituents on the aromatic ring. Furthermore, a number of 3-phenylpropanoates and 3-phenylacrylates were compared in search of a potential effect of conjugation. In addition, methyl, ethyl, propyl, and butyl esters of selected 3-phenylpropanoic and 3-phenylacrylic acids were included to determine the importance of the parent alcohol part of the esters. Finally, we compared the results from structural alterations on the antifeedant activity of the phenylpropanoids with the results from a similar previous study on benzoic acid derivatives (Unelius et al. 2006) in search for similarities and differences in selectivity.

Methods and Materials

Collection and maintenance of weevils Both sexes of *H. abietis* were collected during spring migration at a saw mill in southern Sweden where they landed in large numbers. After collection, weevils were stored in darkness at 10°C and provided with fresh branches of Scots pine, *Pinus sylvestris*, as food. These storage conditions interrupted the reproductive development so that females did not begin ovipositing until about a week after they had been transferred to the experimental conditions, i.e., a light regime of L18/D6 at 22°C. This transfer was made about 10 d before the insects were used in the following bioassay.

Laboratory bioassay Compounds were tested for their antifeedant effect on *H. abietis* by means of a two-choice laboratory bioassay (Bratt et al. 2001; Legrand et al. 2004; Borg-Karlson et al. 2006; Unelius et al. 2006). For each test, 40 pine weevils (20 females+20 males) were starved for 24 hr before the test period. Each weevil was placed in a Petri dish (142 mm diameter) and provided with a Scots pine twig (prepared as described below) placed on a moistened filter paper. One day before the test, the twigs were wrapped in aluminum foil, and two holes (diameter 5 mm; 25 mm apart) were punched in the foil with sharp-edged metal rings. After the removal of the aluminum foil inside the rings, one of the two areas was treated with 100 μl of a 50- or 5-mM methyl acetate solution of the compound to be tested, and the other was treated with the same amount of pure solvent as control. When the solvent had evaporated on the following day, the metal rings were removed and the bioassay started. After 24 hr, the proportion of treated bark and control area of each test

twig that had been consumed was recorded. There was generally no significant difference in response between the sexes, and the data presented have been pooled.

The effects of the various treatments are described by two alternative antifeedant indices (AFI) (Blaney et al. 1984) with the general formula: $100\times(C-T)/(C+T)$. The two variants are: (1) antifeedant index, area (AFIa) where C represents the mean area of the control surfaces consumed and T represents the mean area of the treated surfaces consumed and (2) antifeedant index, number (AFIn) where C represents the number of the control surfaces with feeding scars and T represents the number of the treated surfaces with feeding scars.

Thus, AFIa is a measure that captures the reduction in feeding, whereas AFIn is a measure of the frequency of complete inhibition of the initiation of feeding. The two indices were fairly well correlated, but AFIa tended to be higher than AFIn because the antifeedant substances generally affected both the initiation of feeding and the amount of bark consumed if feeding commenced. For both indices, positive values (up to a maximum of 100) reflect an antifeedant effect, whereas negative ones (down to a minimum of −100) indicate a stimulant effect.

Test chemicals The origins of the compounds tested are given in Tables 1, 2, and 3. Final purities ranged from 96% to 99%. When necessary, compounds were purified by preparative chromatography (Baeckström et al. 1987) or flash chromatography on silica gel (Merck 60, 0.040–0.063 mm, Darmstadt, Germany). The tested esters were synthesized by methods A–F (see "Synthesis" below), purchased from Sigma-Aldrich Sweden AB, Sweden or obtained from previous work by H. Erdtman and T. Norin at KTH Chemistry, Organic Chemistry, Stockholm. All cinnamic acid derivatives had the (*E*)-configuration.

Synthesis Commercial phenylpropanoic and phenylacrylic acids were either esterified by using methods A or B or the esters were formed according to methods C–F.

Method A: Acid-catalyzed esterification from corresponding commercial phenylpropanoic or -phenylacrylic acids. A typical procedure is given for the synthesis of methyl 3-(4-methylphenyl)acrylate: 3-(4-methylphenyl)acrylic acid (1.2 mmol) was dissolved in methanol (11 ml). Concentrated sulfuric acid (three drops) was added. The mixture was refluxed for 4 hr, concentrated with a rotary evaporator, diluted with water, and extracted with ethyl ether. The ether phase was washed with Na_2CO_3 (aq), dried over $MgSO_4$, and evaporated to give white crystals (1.15 mmol, 93%).

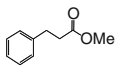
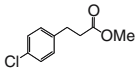
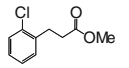
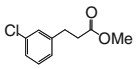
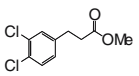
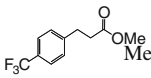
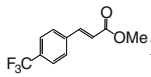
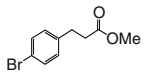
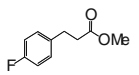
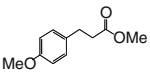
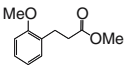
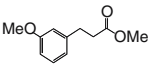
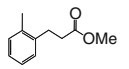
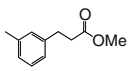
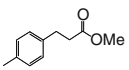
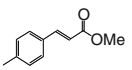
Method B: Preparation of esters by reaction of the carboxylic acid with dicyclohexylcarbodiimide (DCC), 4-methylaminopyridin (DMAP), and methanol in CH_2Cl_2. A typical example is the synthesis of methyl-3-(4-(dimethylamino)phenyl)acrylate: 3-(4-(dimethylamino)phenyl) acrylic acid (3.15 mmol) and DCC (3.67 mmol) were dissolved in methanol (35 ml). A solution of DMAP (1.23 mmol) in CH_2Cl_2 (5 ml) was added, and the reaction mixture was refluxed for 2 hr, concentrated with a rotary evaporator, dissolved in CH_2Cl_2, filtered, washed with water, and dried over $MgSO_4$. Evaporation of the solvent and purification by silica gel chromatography yielded the product (2.44 mmol, 77%) as yellow crystals.

Method C: Preparation of propanoates by catalytic hydrogenation of acrylate analogs (Vogel 1989). A typical example is the formation of methyl 3-(4-(dimethylamino)phenyl)propanoate: Methyl 3-(4-(dimethylamino)phenyl)acrylate (1.32 mmol) was dissolved in methanol (30 ml). To the solution, 39 mg Pd/C (10%) were added, and the reaction mixture was set under an atmosphere of hydrogen. When the reaction was completed after 45 min, the catalyst was filtered off, and the solvent evaporated. Methyl 3-(4-(dimethylamino) phenyl)propanoate was obtained (0.83 mmol, 63%) as slightly yellow crystals.

Method D: Preparation of propanoates by *O*-alkylation of hydroxyl-substituted analogs (Legrand et al. 2004): Isopropyl 3-(4-methoxyphenyl)propanoate was obtained by reacting isopropyl 3-(4-hydroxyphenyl)propanoate with methyl iodide and potassium carbonate in acetone. Methyl 3-(4-butoxyphenyl)propanoate was obtained by reacting methyl 3-(4-hydroxyphenyl)propanoate with potassium hydroxide and butyl iodide. Methyl 3-(3,4-dimethoxyphenyl)propanoate was obtained by reacting methyl 3-(3,4-dihydroxyphenyl)propanoate with sodium hydride and methyl iodide in THF according to the standard procedure (Vogel 1989).

Method E: Preparation of 3-phenylacrylates by Knoevenagel reaction (Harwood et al. 1998) followed by acid-catalyzed esterification. A typical example is the synthesis of methyl 3-(4-ethylphenyl)acrylate: Malonic acid (10.4 mmol) was dissolved in pyridine (3 ml) with heating. 4-Ethylbenzaldehyde (10.2 mmol) and a catalytic amount of piperidine (10 drops) were added while stirring. The reaction mixture was refluxed until the production of carbon dioxide

Table 1 Structures of mono-substituted phenylpropanoids and physical properties - Hansch parameter values for substituents on the aromatic rings (Hansch et al. 1973; Topliss 1972; Swain and Lupton 1968)

Entry	Structure	Name of compound	σ	π	F	R	MR	MW
1		Methyl 3-phenylpropanoate [a]	0	0	0	0	0	1
2		Methyl 3-(4-chlorophenyl)propanoate [a]	0.23	0.71	0.41	-0.15	6.03	35
3		Methyl 3-(2-chlorophenyl)propanoate [a]	N/A	0.71	0.41	-0.15	6.03	35
4		Methyl 3-(3-chlorophenyl)propanoate [a]	0.37	0.71	0.41	-0.15	6.03	35
5		Methyl 3-(3,4-dichlorophenyl)propanoate [a]	0.60	1.42	0.82	-0.30	12.1	71
6		Methyl 3-(4-trifluoromethylphenyl)propanoate [b]	0.54	0.88	0.38	0.19	5.02	69
7		Methyl 3-(4-trifluoromethylphenyl)acrylate [a]	0.54	0.88	0.38	0.19	5.02	69
8		Methyl 3-(4-bromophenyl)propanoate [a]	0.23	0.86	0.44	-0.17	8.88	80
9		Methyl 3-(4-fluorophenyl)propanoate [a]	0.06	0.14	0.43	-0.34	0.92	19
10		Methyl 3-(4-methoxyphenyl)propanoate [c]	-0.27	-0.02	0.26	-0.51	7.87	31
11		Methyl 3-(2-methoxyphenyl)propanoate [a]	N/A	-0.02	0.26	-0.51	7.87	31
12		Methyl 3-(3-methoxyphenyl)propanoate [a]	0.12	-0.02	0.26	-0.51	7.87	31
13		Methyl 3-(2-methylphenyl)propanoate [a]	N/A	0.56	-0.04	-0.13	5.65	15
14		Methyl 3-(3-methylphenyl)propanoate [a]	-0.07	0.56	-0.04	-0.13	5.65	15
15		Methyl 3-(4-methylphenyl)propanoate [a]	-0.17	0.56	-0.04	-0.13	5.65	15
16		Methyl 3-(4-methylphenyl)acrylate [a]	-0.17	0.56	-0.04	-0.13	5.65	15

[a] prepared from the corresponding carboxylic acids by refluxing in the alcohol with H_2SO_4 as a catalyst: Method A

[b] prepared by catalytic hydrogenation of acrylates: Method C.

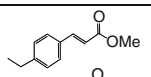
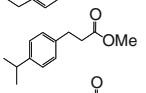
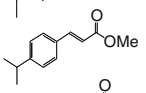
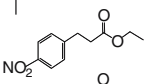
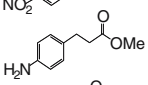
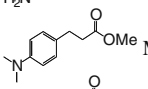
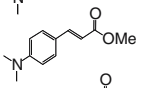
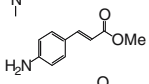
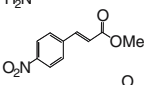
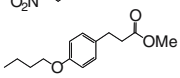
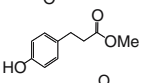
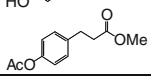
[c] obtained from previous work by H. Erdtman and T. Norin at the Dep. of Organic Chemistry, KTH, Stockholm.

abated. HCl (aq, 2 M, 10 ml) was added, and the precipitate formed was filtered off by suction and washed with HCl (aq, 2 M, 10 ml), water (10 ml), and hexane (10 ml) and dried *in vacuo* to yield methyl 3-(4-ethylphenyl)acrylic acid (7.8 mmol, 76%). The carboxylic acid was esterified according to method A.

Method F: Methyl 3-(4-acetyloxyphenyl)propanoate was prepared from methyl 3-(4-hydroxyphenyl) propanoate according to the standard procedure (Vogel 1989).

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

Table 1 (continued)

Entry	Structural formula	Compound	σ	π	F	R	MR	MW
17		Methyl 3-(4-ethylphenyl)acrylate [d]	-0.15	1.02	-0.05	-0.10	10.3	29
18		Methyl 3-(4-isopropylphenyl)propanoate [b]	-0.15	1.53	-0.05	-0.10	15.0	43
19		Methyl 3-(4-isopropylphenyl)acrylate [b]	-0.15	1.53	-0.05	-0.10	15.0	43
20		Ethyl 3-(4-nitrophenyl)propanoate [b]	0.78	-0.28	0.67	0.16	7.36	46
21		Methyl 3-(4-aminophenyl)propanoate [e]	-0,66	-1,23	0	-0.68	5.42	16
22		Methyl 3-(4-dimethylaminophenyl)propanoate [e]	-0.83	0.18	0.10	-0.92	15.6	44
23		Methyl 3-(4-dimethylaminophenyl)acrylate [f]	-0.83	0.18	0.10	-0.92	15.6	44
24		Methyl 3-(4-aminophenyl)acrylate [b]	-0,66	-1,23	0	-0.68	5.42	16
25		Methyl 3-(4-nitrophenyl)acrylate [b]	0.78	-0.28	0.67	0.16	7.36	46
26		Methyl 3-(4-butoxyphenyl)propanoate [g]	-0.32	1.55	0.25	-0.55	21.7	73
27		Methyl 3-(4-hydroxyphenyl)propanoate [h]	-0.37	-0.67	0.29	-0.64	2.85	17
28		Methyl 3-(4-acetyloxyphenyl)propanoate [i]	0.31	-0.64	0.41	-0.07	12.5	59

[d] prepared via a Knoevenagel condensation: Method E.
[e] prepared by catalytic hydrogenation of cinnamates: Method C.
[f] prepared from the carboxylic acid by reaction with DCC, DMAP and methanol in CH_2Cl_2: Method B.
[g] prepared by O-alkylation of hydroxy-substituted analogs: Method D.
[h] purchased from SigmaAldrich Co, Sweden.
[i] prepared by O-acetylation of hydroxy-substituted analogs: Method F.

Spectroscopy H NMR (400 or 250 MHz) and C NMR (100 or 63 MHz) spectra were recorded on Varian 400, Bruker 400, or Bruker 250 instruments using the solvent signals, $CDCl_3$ or CD_3OD, as internal standards.

The Topliss scheme The first substance in the Topliss scheme possesses an unsubstituted aromatic ring (or a phenylic compound with one or more substituents not being changed over the time of the study) (Topliss 1972). In the modified Topliss scheme used, we started with methyl 3-phenylpropanoate (Fig. 1). The analogs were tested at 50 mM concentration in methyl acetate with the solvent as control treatment. As the AFI values for many substances were close to 100 at 50 mM, new tests were conducted at 5 mM concentration. The Topliss scheme was used continuously in the dynamic experimental plan, i.e., after the synthesis of each compound and after the evaluation of the test results from bioassays, the scheme was consulted for the choice of compound next to be synthesized and tested. Finally, analogs from all positions of the scheme were synthesized and tested to critically evaluate the method.

Hansch parameters The Hansch equation (Eq. 1) contains a number of parameters that reflect different properties of substituents on aromatic rings (Hansch and Deutsch 1966), such as hydrophobicity (π), electronic effects (σ, F, and R) and steric factors (molar refractivity [MR] and molecular weight [MW]). The relation between steric parameters can be found in the Lorentz–Lorentz equation (Eq. 2). One expression for the relationship between electronic effects is also shown (Eq. 3).

$$\mathrm{Log}(1/C) = k_1(\log\pi)^2 + k_2\log\pi + k_3\sigma + k_4\mathrm{MR} + k_5 \quad (1)$$

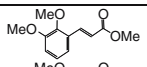
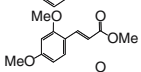
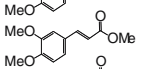
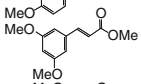
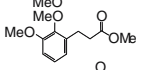
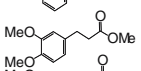
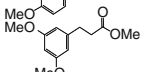
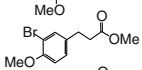
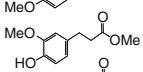
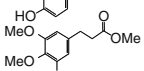
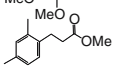
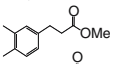
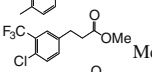
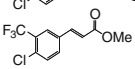
$$\mathrm{MR} = \mathrm{MW}\left(n^2-1\right)/d\left(n^2+2\right)\left[\mathrm{cm}^3/\mathrm{mol}\right] \quad (2)$$

$$R = \sigma - k_6F \quad (3)$$

C is the concentration required for biological activity, k_1–k_6 are the system-dependent constants, n is the refraction index, and d is the density.

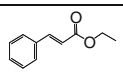
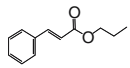
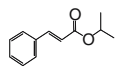
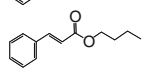
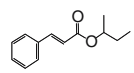
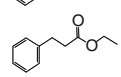
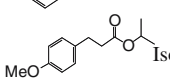
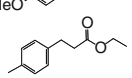
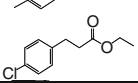
In this study, the hydrophobic parameter used was π, derived from log P values (Hansch et al. 1973). Three

Table 2 Structures of disubstituted and trisubstituted phenylpropanoids and physical properties - Hansch parameter values for substituents on the aromatic rings (Hansch et al. 1973; Topliss 1972; Swain and Lupton 1968)

Entry	Structural formula	Compound	σ	π	F	R	MR	MW
29		Methyl 3-(2,3-dimethoxyphenyl)acrylate [a]	N/A	-0.04	0.52	-1.02	15.7	62
30		Methyl 3-(2,4-dimethoxyphenyl)acrylate [a]	N/A	-0.04	0.52	-1.02	15.7	62
31		Methyl 3-(3,4-dimethoxyphenyl)acrylate [a]	-0.15	-0.04	0.52	-1.02	15.7	62
32		Methyl 3-(3,5-dimethoxyphenyl)acrylate [a]	0.24	-0.04	0.52	-1.02	15.7	62
33		Methyl 3-(2,3-dimethoxyphenyl)propanoate [b]	N/A	-0.04	0.52	-1.02	15.7	62
34		Methyl 3-(3,4-dimethoxyphenyl)propanoate [c]	-0.15	-0.04	0.52	-1.02	15.7	62
35		Methyl 3-(3,5-dimethoxyphenyl)propanoate [b]	0.24	-0.04	0.52	-1.02	15.7	62
36		Methyl (3-bromo-4-methoxyphenyl)propanoate [a]	0.12	0.84	0.70	-0.68	16.8	111
37		Methyl 3-(4-hydroxy-3-methoxyphenyl)propanoate [a]	-0.25	-0.69	0.55	-1.15	10.7	48
38		Methyl 3-(3,4,5-trimethoxyphenyl)propanoate [a]	-0.03	-0.06	0.78	-1.53	23.6	93
39		Methyl 3-(2,4-dimethylphenyl)propanoate [a]	N/A	1.12	-0.08	-0.26	11.3	30
40		Methyl 3-(3,4-dimethylphenyl)propanoate [a]	-0.24	1.12	-0.08	-0.26	11.3	30
41		Methyl 3-(4-chloro-3-trifluoromethylphenyl)propanoate [b]	0.66	1.59	0.79	0.04	11.0	104
42		Methyl 3-(4-chloro-3-trifluoromethylphenyl)acrylate [a]	0.66	1.59	0.79	0.04	11.0	104

[a] prepared from the corresponding carboxylic acids by refluxing in the alcohol with H2SO4 as a catalyst: Method A.
[b] prepared by catalytic hydrogenation of cinnamates: Method C.
[c] prepared by O-alkylation of hydroxy-substituted analogs: Method D.

Table 3 Structures of phenylpropanoids (variations in the parent alcohol parts) and physical properties - Hansch parameter values for substituents on the aromatic rings (Hansch et al. 1973; Topliss 1972; Swain and Lupton 1968)

Entry	Structural formula	Compound	σ	π	F	R	MR	MW
43		Ethyl cinnamate [a]	0	0	0	0	0	1
44		Propyl cinnamate [a]	0	0	0	0	0	1
45		Isopropyl cinnamate [a]	0	0	0	0	0	1
46		Butyl cinnamate [a]	0	0	0	0	0	1
47		2-Butyl cinnamate [a]	0	0	0	0	0	1
48		Ethyl 3-phenylpropanoate [a]	0	0	0	0	0	1
49		Isopropyl 3-(4-methoxyphenyl)propanoate [b]	-0.27	-0.02	0.26	-0.51	7.87	31
50		Ethyl 3-(4-methylphenyl)propanoate [a]	-0.17	0.56	-0.04	-0.13	5.65	15
51		Ethyl 3-(4-chlorophenyl)propanoate [a]	0.23	0.71	0.41	-0.15	6.03	35

[a] prepared from the corresponding carboxylic acids by refluxing in the alcohol with H2SO4 (catalyst): Method A.
[b] prepared by O-alkylation of hydroxyl-substituted analogs: Method D.

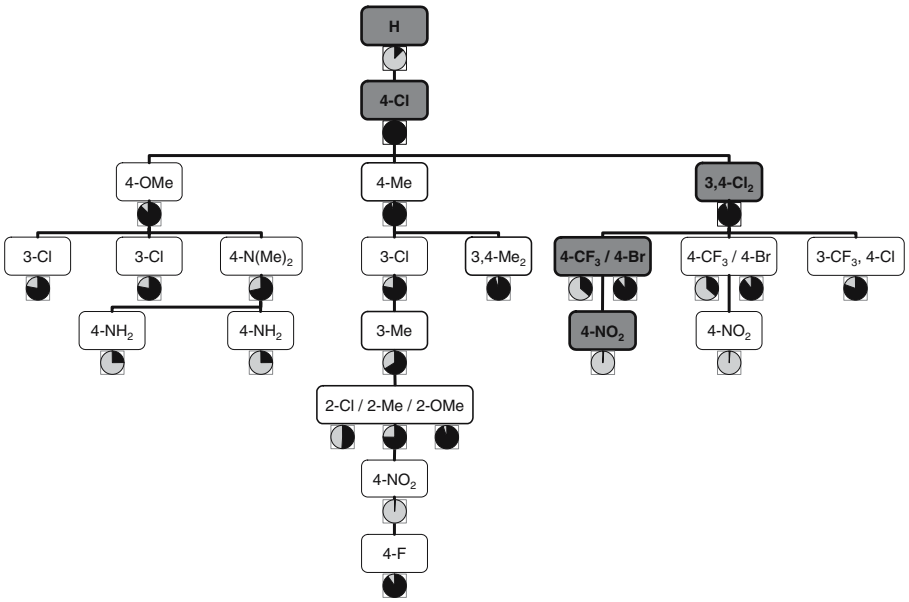


Fig. 1 The complete modified Topliss scheme used in this study. Substituents on the aromatic ring are shown for each analog. From each "parent" compound (from the top of the scheme) the choice of the next analog is shown, depending on the result of the previous test. If the potency increased for the latest tested compound, the next compound to be tested is the one to the right in the next lower level of the scheme, i.e., first, the unsubstituted compound and the 4-chloro-substituted compound is tested, and if the latter compound is more active, the 3,4-dichloro analog is tested next. If the activity is equally high, the analog straight below is tested, i.e., if the 4-Cl analog is equally active as the 4-H, the 4-Me analog is tested next. If the activity is lower, the analog below to the left is tested, i.e., if the 4-Cl analog is less active than the unsubstituted compound, the 4-OMe analog is tested next. Our results directed us to conduct the test series indicated by *shaded, bold boxes*. The results (AFIn) are shown in circle diagrams below each analog (*filled black circle* AFIn=100, *empty gray circle* AFIn=0; all tests carried out at 50 mM)

different electronic parameters were applied: σ, F, and R. The widely used σ parameter has the disadvantage that the resonance effect contributes for metasubstituted aromatic rings (σ values for orthosubstituted compounds have not been included), whereas for parasubstituted systems, the field factors affect the values of σ (Swain and Lupton 1968). For the parameters F and R, the field effects (F) and resonance effects (R) are separated. The steric parameters used to measure the "bulk" of the molecules were molecular weight (MW) and molar refractivity (MR). MR differs from MW in having an electronic contribution, as it is directly proportional to polarizability (Eq. 2; Hansch et al. 1973). The values of all parameters used in this study are available in the literature (Hansch and Deutsch 1966; Swain and Lupton 1968 and Hansch et al 1973).

Results

Evaluation of the Topliss method for finding potent methyl 3-phenylpropanoate antifeedants To determine fully the usability of this approach, the scheme was initially followed step by step (as was intended in Topliss' method); but in a second phase of the study, all compounds found in all branches of the scheme were obtained and compared.

The investigation started with methyl 3-phenylpropanoate (entry 1, Table 1). The first analog to be tested was the more hydrophobic and electron-withdrawing 4-chloro compound (entry 2, Table 1), which was more active than the unsubstituted compound. The next analog to be tested according to the scheme was the 3,4-dichloro compound (entry 5, Table 1), which was even more hydrophobic, and the ring was more electron-poor. This analog showed similar or slightly lower activity. The 4-CF_3 analog (entry 6, Table 1), which did not have a metasubstituent and was less lipophilic than 3,4-Cl but more lipophilic than 4-Cl, was tested next and was less active than the previous one. The 4-Br analog (entry 8, Table 1), an alternative to 4-CF_3, also showed low activity. Finally, the analog with the less lipophilic but more electron-withdrawing 4-NO_2 substituent, can be found in the bottom of scheme 1. Ethyl 3-(4-nitrophenyl)propanoate (entry 20, Table 1) was tested instead of methyl 3-(4-nitrophenyl)propanoate but the results can be compared with the results from ethyl 3-phenylpropanoate (entry 48, Table 3), and it is obvious that nitro analogs have relatively low activities (Fig. 2a and Table 1).

To find out if the Topliss method generated the optimal antifeedant, we decided to investigate all three branches in parallel.

Following the right branch of the Topliss scheme, all compounds except methyl 3(4-chloro-3-trifluoromethyl-phenyl)propanoate (entry 41, Table 2) were tested, as the scheme was followed systematically. This compound was less active than the analogs substituted with 4-Cl (entry 2,

Table 1), 3,4-Cl (entry 5, Table 1), and 4-Br (entry 8, Table 1) but more active than the 4-CF_3 (entry 6, Table 1) and 4-NO_2 (entry 20, Table 1) analogs (Fig. 2a).

In the center branch, the 4-methyl (entry 15, Table 1) and 3,4-dimethyl analogs (entry 40, Table 2) were tested next. Further down this branch, steric factors were examined by testing the 2- and 3-substituted analogs, i.e., the 3-chloro (entry 4, Table 1) and the 2-chloro (entry 3, Table 1), 2-methyl (entry 13, Table 1) and 2-methoxy (entry 11, Table 1) analogs. These compounds generally had lower activity than the 4-substituted analogs but the electronic factors did not seem to greatly affect the activity. The 4-F analog (entry 9, Table 1) has similar values of the Hansch parameters but showed higher potency than the parent unsubstituted methyl 3-phenylpropanoate (Fig. 2b).

For the left branch of the scheme, the choices after the electron-donating 4-methoxy analog (entry 10, Table 1) were other more electron-donating compounds: the 4-N$(Me)_2$ (entry 22, Table 1) and the 4-NH_2 (entry 21, Table 1) analogs. The dimethylamino compound had similar potency compared with the 4-methoxy compound, whereas the amino compound was much less potent (Fig. 2c). As a hypothesis, the polarity of the amino group is the cause of the low antifeedant activity.

To be able to better compare the most potent antifeedants, which all had AFI values close to 100, additional tests were carried out at 5 mM concentration. The results from these tests showed a stronger decrease in activity at the lower concentration for the methoxy analogs than for the analogs with chloro, bromo, fluoro, and methyl substituents (Fig. 3).

As potent antifeedants were found in all branches of the Topliss scheme and not preferentially among the analogs obtained when the proposed route was followed, the Topliss approach was abandoned. Consequently, we continued our survey of the SARs of phenylpropanoids by using other methods.

Effect of the position of substituents on the aromatic ring To see whether substituents on the aromatic ring should be situated in the ortho, meta, or para position for optimal activity, tests were performed for all monosubstituted isomers of the methyl (entries 13–15, Table 1), chloro (entries 2–4, Table 1), and methoxy (entries 10–12, Table 1) analogs (Fig. 3a). For the methyl-substituted compounds, the difference in position did not greatly affect the potency, neither on AFIn nor AFIa. For the monochlorinated compounds, there was a larger difference in activity in the order para>meta>ortho. For the methoxy analogs, the metasubstituted and parasubstituted compounds showed similar potency in all tests, whereas it is interesting to note that the activity of the orthosubstituted analog was almost nil at 5 mM (Fig. 3a).

Effects of unsaturation and conjugation To understand the importance of conjugation to the carbonyl group in the phenylpropanoids, several substituted methyl 3-phenylpropanoates were compared with their corresponding 3-phenylacrylates (Fig. 4). Most of the pairs of conjugated (3-phenylacrylates) and nonconjugated compounds (3-phenylpropanoates) had similar activities (Fig. 4). However, when we compared the activities of the analogs with the most electron-withdrawing and electron-donating groups, we found a trend. The activities of the compounds with electron-withdrawing groups (4-CF_3 and 4-NO_2) (entries 6–7, 20, 25, Table 1) were favored by conjugation, whereas the activity of compounds with electron-donating substituents (4-N$(CH_3)_2$ and 4-NH_2) (entries 21–24, Table 1) decreased for the conjugated analogs (Fig. 4b).

Comparison of disubstituted and trisubstituted compounds We also investigated whether it was possible to improve the activity by adding more substituents to the aromatic ring and by optimizing the substitution pattern. Consequently, several compounds with two or three substituents were tested (Fig. 3b). Different substitution patterns were compared for dimethoxy- and trimethoxy-substituted (entries 33–35, 38, Table 2) methyl 3-phenylpropanoates. For the dimethoxy-substituted compounds, there was a strong effect favoring 2,3- and 3,5-substitution over 3,4- and 3,4,5-substitution. The 2,3- and 3,5-disubstituted methoxy analogs were slightly less active compared with the monosubstituted methoxy analogs (Fig. 3b). For disubstituted methyl analogs (entries 39–40, Table 2), the 2,4-analog was slightly more potent than the 3,4-analog, but the difference was minor. The disubstituted methyl compounds were approximately as potent as the monosubstituted analogs (Fig. 3c). Furthermore, methyl 3-(4-ethylphenyl)acrylate (entry 17, Table 1) was tested to establish whether the larger and more hydrophobic ethyl group alone could enhance the activity, but this compound was less active than the ones previously tested.

The 3,4-dichlorinated analog (entry 5, Table 1) was tested and compared with the monohalogenated analogs. It showed similar potency as the 3-chloro, (entry 4, Table 1) but slightly less potency than the 4-chloro compound (entry 2, Table 1).

A series of dimethoxy-substituted 3-phenylacrylates (entries 29–32, Table 2) were also tested for the influence of substitution pattern on activity. The order of reactivity between combinations of substituents was 2,3>2,4≫3,5>3,4 (Fig. 4a). These results corresponded well with the results from the dimethoxyphenylpropanoates above.

Activity of phenylpropanoates with mixed substituents - Three analogs with mixed substituents were added to the

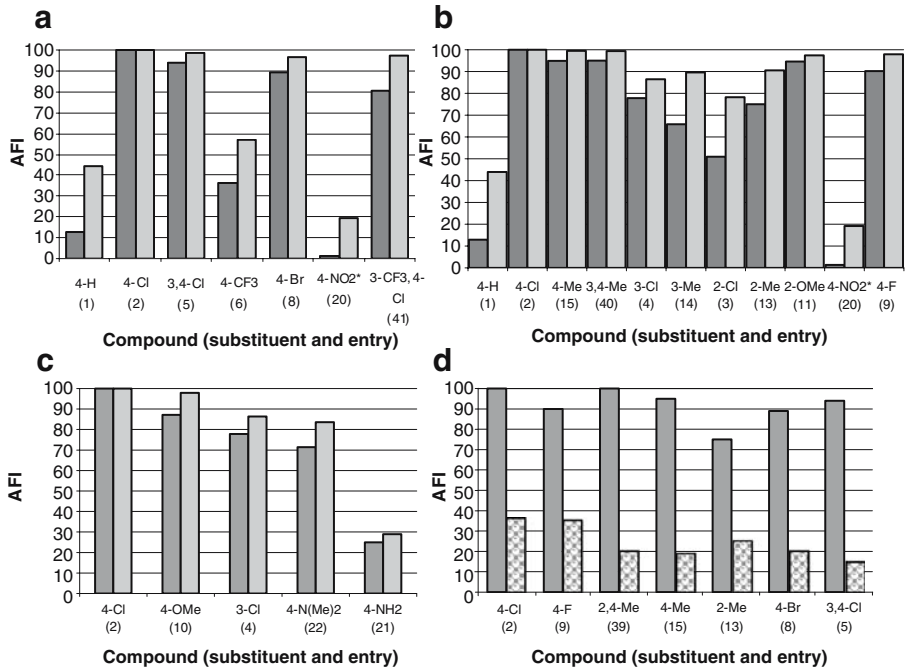


Fig. 2 AFI for substituted 3-phenylpropanoates in the Topliss scheme. AFIn is represented by *dark columns* and AFIa by *light columns*. Substituent(s) and entry number (in *parentheses*) are given beneath the *x*-axis: **a** the right branch (50 mM concentration), ethyl 3-(4-nitrophenyl)propanoate is denoted by an *asterisk*; **b** the center branch (50 mM concentration), ethyl 3-(4-nitrophenyl)propanoate is denoted by an *asterisk*; **c** the left branch (50 mM concentration); **d** AFIn for the *most potent* compounds from the Topliss scheme tested at 50 mM (*dark columns*) and 5 mM concentrations (*dotted columns*)

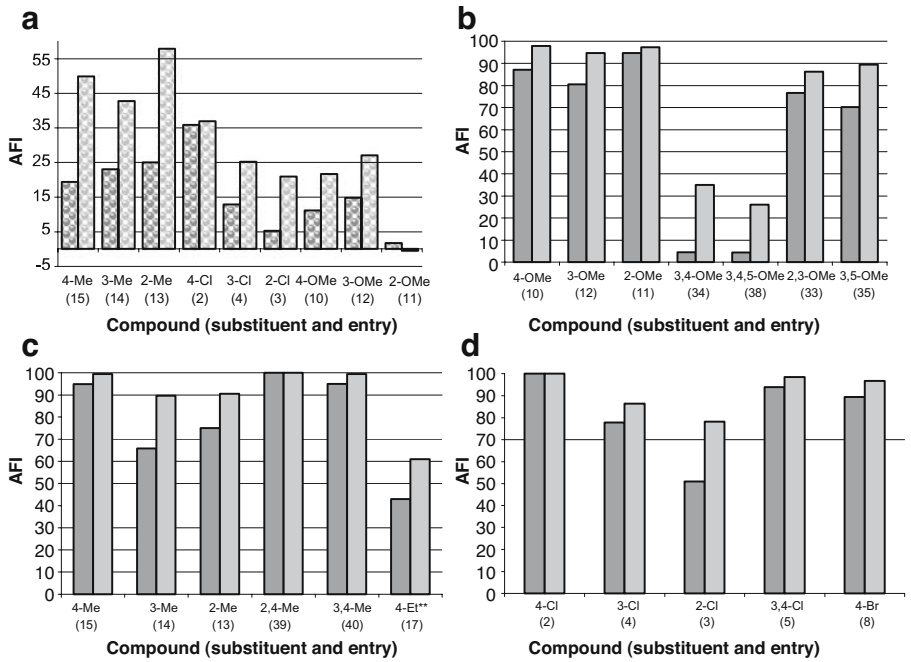


Fig. 3 AFI for substituted 3-phenylpropanoates. AFIn is represented by *dark columns* and AFIa by *light columns*. Substituent(s) and entry number (in *parentheses*) are given beneath the *x*-axis: **a** methyl-, chloro-, and methoxy-substituted analogs (5 mM concentration, *dotted columns*); **b** methoxy-substituted analogs (50 mM concentration); **c** methyl-substituted analogs (50 mM concentration), methyl 3-(4-ethylphenyl)acrylate is denoted by *two asterisks*; **d** chloro- and bromo-substituted analogs (at 50 mM concentration)

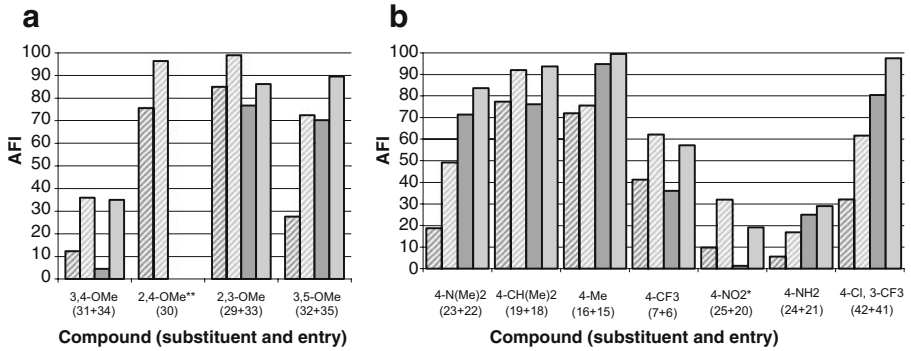


Fig. 4 AFI for methyl 3-phenylacrylates (acr; *striped columns*) and methyl 3-phenylpropanoates (pro; *plain columns*) at 50 mM concentration. AFIn is represented by *dark columns* and AFIa by *light columns*. Substituent(s) and entry number (in *parentheses*) are given beneath the *x*-axis. Each group of compounds with identical substitution pattern are shown in the following order: AFIn(acr), AFIa(acr), AFIn(pro), and AFIa(pro). Ethyl 3-(4-nitrophenyl)propanoate tested instead of methyl 3-(4-nitrophenyl)propanoate is denoted by an *asterisk*; 3-phenylpropanoate analog not tested is denoted by *two asterisks*. **a** Methoxy-substituted analogs; **b** analogs with substituents other than methoxy

test series. These were the 3-bromo-4-methoxy (entry 36, Table 2), 4-chloro-3-trifluoromethyl (entry 41, Table 2), and 4-hydroxy-3-methoxy analogs (entry 37, Table 2). The first compound was less active than the corresponding mono-substituted analogs ((3-Br, 4-OCH_3) vs. 4-Br and 4-OCH_3). The (3-CF_3, 4-Cl) analog was less potent than 4-Cl but more potent than 4-CF_3, and the (3-OCH_3, 4-OH) analog was a more active antifeedant than 4-OH (Fig. 5a). Although not all possible variations were tested, the conclusion from our test series was that no beneficial effects could be found by mixing different substituents on the aromatic ring.

Effect of the parent alcohol part of the esters on antifeedant activity of phenylpropanoids The methyl (entry 10, Table 1) and isopropyl (entry 49, Table 3) esters of 3-(4-methoxyphenyl)propanoate and five alkyl cinnamates (ethyl-, *n*-propyl-, isopropyl-, *n*-butyl-, and 2-butylcinnamate) (entries 43–47, Table 3) were compared to elucidate the effect of the parent alcohol of the esters. All compounds were tested at 50 mM concentration. Isopropyl cinnamate was the most potent of the cinnamates (Fig. 5b) while methyl 3-(4-methoxyphenyl)propanoate was more potent than the corresponding isopropyl ester analog (Fig. 5c). The butyl esters were much less active in all tests, and therefore, no esters derived from higher alcohols were tested.

Furthermore, the methyl and ethyl esters of 3-phenylpropanoic acid, (entry 1, Table 1, vs. entry 48, Table 3), 3-(4-methylphenyl)propanoic acid (entry 15, Table 1, vs. entry 50, Table 3) and 3-(4-chlorophenyl)propanoic acid (entry 2, Table 1, vs. entry 51, Table 3) were compared. Apparently, no major difference in activity between methyl and ethyl esters were detectable except for the unsubstituted 3-phenylpropanoate where the methyl ester was considerably less active compared to the ethyl ester (Fig. 5c).

Influences by Hansch parameters By comparing Hansch parameters in relation to antifeedant activity for a series of substituted methyl 3-phenylpropanoates, the effect of each parameter was analyzed. The aim was to find the importance of each parameter and thus the properties of the substituents that affect antifeedant activity. In total, 29 methyl 3-phenylpropanoates and 11 3-phenylacrylates were compared.

The Hansch parameters that were examined for correlations to antifeedant activity were σ, π, F, R, MR, and MW (Tables 1, 2, and 3). For the electronic parameter σ and the field effect (F), there was no apparent correlation to antifeedant potency. A similar result was found after analyses of the steric parameters (MR and MW), i.e., small and light molecules as well as bulky and heavy compounds showed good antifeedant activity. For the lipophilicity parameter (π), there was a correlation with antifeedant potency. If π was negative ($\pi<-0.1$), the activity was, on average, lower (Fig. 6a). The resonance factor R also showed a correlation to AFI: No compound with good antifeedant potency had $R<-0.5$ (Fig. 6b). For 3-phenylacrylates, the results were similar.

Comparison with benzoate antifeedants In a previous study, we measured the AFI of a large number of benzoates (Unelius et al. 2006). Methoxy-substituted benzoates showed high antifeedant activity. Therefore, the following analogous phenylpropanoids were tested in addition to the compounds already discussed: Methyl 3-(4-butoxyphenyl) propanoate, (entry 26, Table 1), methyl 3-(4-hydroxyphenyl) propanoate (entry 27, Table 1), methyl 3-(4-acetyloxyphenyl)propanoate (entry 28, Table 1), and methyl 3-(4-hydroxy-3-methoxyphenyl)propanoate (entry 37, Table 2). In agreement with the results from the benzoate study, the methoxy analogs (for example, 3-(4-methoxyphenyl)prop-

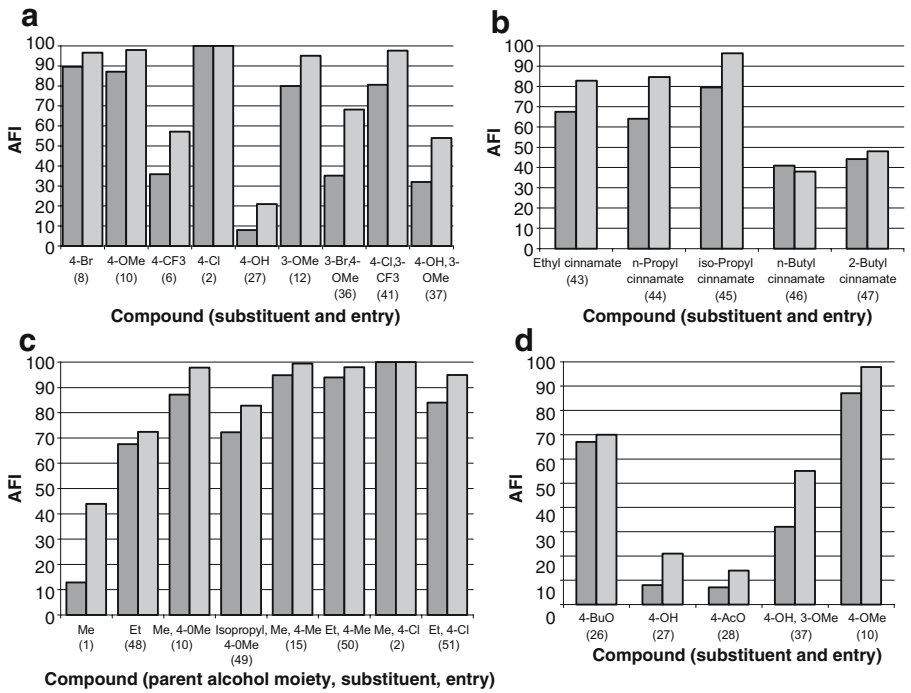


Fig. 5 AFI for 3-phenylpropanoids (at 50 mM concentration). AFIn is represented by *dark columns* and AFIa by *light columns*. Substituent (s) and entry number (in *parentheses*) are given beneath the *x*-axis: **a** methyl 3-phenylpropanoates with mono and mixed substituents; **b** cinnamates with different parent alcohol moieties; **c** 3-phenylpropanoates with different parent alcohol moieties; **d** derivatives of methyl 3-(4-hydroxyphenyl)propanoate

anoate; entry 10, Table 1) were most effective (Fig. 5d), but, in contradiction to the results from the benzoate study, the tested methyl- or halogen-substituted phenylpropanoids had similar or even higher antifeedant activity than the methoxy analogs.

Discussion

When initially applying the Topliss approach in our search for potent antifeedants, the most active compounds happened to appear early in the scheme, so the use of the remaining scheme became redundant. Normal rational design was used, therefore, during the reminder of this SAR study. Nevertheless, the Topliss approach may be a useful tool in other projects within the field of chemical ecology where an effective semiochemical is to be found in a limited time and at low costs.

To correlate the structures of the phenylpropanoids to their antifeedant activity for *H. abietis* was difficult. Apart from the result that highly polar substituents were unfavorable for activity, it was difficult to discriminate any particular factors that affected the antifeedant activity. The position of substituents on the aromatic ring does not appear to be critical for the antifeedant activity–a number of 2-, 3-, and 4-substituted analogs had similar potency. The most potent compounds tested were methyl 3-phenylpropanoates mono-substituted with chloro, fluoro, or methyl groups, and the 3,4-dichlorinated methyl 3-phenylpropanoate.

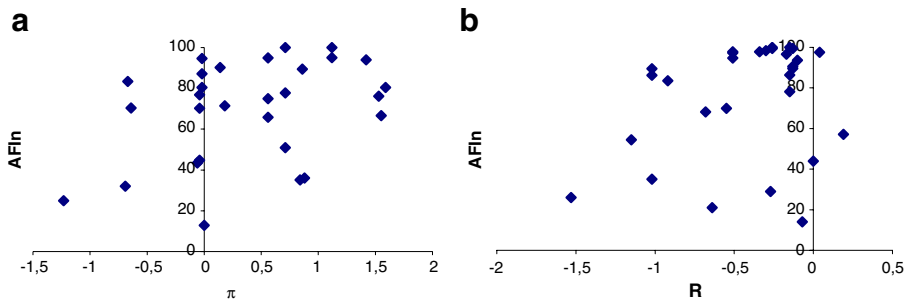


Fig. 6 AFIn for methyl 3-phenylpropanoates (at 50 mM concentration) vs. values of various Hansch parameters: **a** AFIn vs. the hydrophobicity parameter π; **b** AFIn vs. the steric parameter *R*

In a comparison of 3-phenylpropanoates and acrylates, the lower flexibility caused by the double bond in the acrylates may be an important factor that affects interactions with a receptor. Nevertheless, we focused on the electronic effects in our analyses of conjugated vs. nonconjugated analogs. In compounds with electron-withdrawing substituents on the aromatic ring, the antifeedant effect increased with conjugation, and for compounds with electron-donating substituents, the effect decreased for the conjugated analogs. Electron richness in the aromatic ring of the phenylpropanoids may be important for high activity. Alternatively, or additionally, the carbonyl moiety should be deprived of electron density. Eriksson (2006) studied the antifeedant activity of 3-phenylpropenals and 3-phenylpropanals against *H. abietis* and concluded that conjugation does not seem to be critical for activity.

In the comparison between monosubstituted and disubstituted compounds, the disubstituted methyl analogs were slightly less potent than the monosubstituted. The decreased potency cannot be correlated to a negative effect of the increased bulkiness of the compounds as this effect was not seen for the chloro analogs. The magnitude of difference observed (more than tenfold difference) between the disubstituted and trisubstituted methoxy analogs is intriguing. The 3,4- and 3,4,5-substituted saturated compounds were much less active than the 2,3- and 3,5-substituted, which is in agreement with the results from saturated analogs of cinnamic aldehyde (Eriksson 2006). When we compared the antifeedant effect of conjugated acrylate analogs (3,4-OCH_3, 2,4-OCH_3, 2,3-OCH_3, and 3,5-OCH_3), the results differed considerably. In this case, the 2,3- and 2,4-analogs were more potent than the 3,4- and 3,5-analogs, which is contradictory to the results for the corresponding phenylpropenals (Eriksson 2006). Our results indicate that there was an effect by conjugation.

For the compounds with mixed substituents, the (3-Br, 4-OCH_3) analog was less potent than the monosubstituted compounds, which implies that the increased bulk lowered activity. In contrast, the (3-CF_3, 4-Cl) compound was more potent than its monosubstituted analogs.

When esters with different alcohol parts were compared, methyl, ethyl, and isopropyl esters showed potency within the same range, whereas the butyl esters tested showed lower potency. One explanation could be that the bulkiness of the butyl analogs caused the lower antifeedant activity.

The investigation of the relation of the various Hansch parameters to antifeedant activity revealed a correlation to π and R, respectively. For π, a negative value ($\pi<-0.1$) gave a lower AFI. All eight compounds with a more negative F had AFIa<60 at 50 mM concentration. This result was expected as too highly hydrophilic compounds have not been very active in previous investigations of antifeedants for *H. abietis* (Unelius et al. 2006; Sunnerheim et al. 2007). A similar relationship was found for R where compounds having $R<-0.5$ showed low AFIn and AFIa values, i.e., a relatively high resonance potential seems to be necessary. The steric parameters, MR and MW, showed no correlation to antifeedant activity. When the steric factors for each position (ortho, meta, para) on the aromatic ring were correlated to the AFIs, no general trend was found, but the number of compounds tested was limited (10 para, 3 meta and 3 orthomonosubstituted compounds). These results may be indicative of a multireceptor response that causes the antifeedant effect, since small as well as bulky, and light as well as heavy compounds all showed similar antifeedant potency. This multireceptor hypothesis is supported by the fact that most other parameters also did not correlate to AFI.

In the relatively few studies available on the responses of other organisms to phenylpropanoids, there are both similarities and dissimilarities with our results for *H. abietis*. That esters of phenylpropanoids are more active than the corresponding alcohols and acids was shown both in the present study and in studies of derivatives of cinnamic acid as bird repellents (Jacubas et al. 1992; Watkins et al. 1999). An example of a compound with diverging effects between organisms is ethyl 3-(4-nitrophenyl)acrylate, which showed no antifeedant activity against *H. abietis* although it has previously been found to be an oviposition deterrent for the onion fly *Delia antique* (Meigen) (Cowles et al. 1990). Another related compound, cinnamaldehyde, has been proved to act as an antifeedant for grain storage insects (Huang and Ho 1998). A comparison of the phenylpropanoid vs. the benzoate antifeedants of *H. abietis* (Unelius et al. 2006) reveals a number of similarities: Carboxylic acids are generally less effective antifeedants than the corresponding esters, which also is in agreement with the results from the study on *H. abietis* by Eriksson (2006). Furthermore, the length of the alkyl chain in the parent alcohol moiety of the esters should be short, and methoxy-substituted aromatics are more potent than hydroxy-substituted in both classes of substances. On the other hand, the prerequisites of the substitution pattern for good antifeedant activity seem to differ between benzoates and phenylpropanoids. For example, the best benzoate is the 2,4-dimethoxy analog, whereas the corresponding 2,4-dimethoxy-phenylpropanoids have rather low activity, and the most effective propanoids, the halogen- or methyl-substituted compounds, have very low AFIs as benzoate analogs.

Among the 51 compounds tested in this study, a large number of potent antifeedants were found. A majority of the compounds tested was more effective than the lead compound, ethyl cinnamate, which has been identified in the inner bark of *Pinus contorta* (Bratt et al. 2001). The most potent compounds were methyl 3-phenylpropanoates monosubstituted with chloro, fluoro, or methyl groups on the aromatic

ring, and the 3,4-dichlorinated methyl 3-phenylpropanoate. These compounds are good candidates for further studies in the laboratory and in the field. A pool of over 20 highly active phenylpropanoid compounds provides many possibilities to develop mixtures of compounds that could potentially be even more effective than the single compounds tested so far. Field assays that evaluate chemical and physical properties, such as volatility and stability to resist water and UV radiation, are required to establish the effectiveness of these compounds for conifer seedling protection under field conditions.

Our findings are probably a consequence of effects from the stimuli of several taste and odor receptors processed by the insect brain. Earlier investigations (Wibe et al. 1996, 1997, 1998) have described the response of numerous receptors in the antennae of *H. abietis*, of which some are highly specific and some more generally tuned to olfactory stimuli, within groups of monoterpenes and aromatic compounds. Thus, it is likely that several receptor types are activated during feeding and that each type is tuned to molecules with different structural properties. The results from a study of structural analogs of naphthoquionones as feedants/antifeedants for the larvae of the Mexican bean beetle, *Epilachna varivestis*, are as intriguing as ours. A complex interplay of electronic, steric, electrochemical, and positional requirements was found that affected the feeding response (Weissenberg et al. 1997).

The ecological background to why feeding of *H. abietis* is affected by derivatives of phenylpropanoates is still largely unknown. Although it is possible that ethyl cinnamate is partly responsible for the fact that *H. abietis* feeds less on the bark of *P. contorta* than of *P. sylvestris* (Bratt et al. 2001), this is at least not specifically a substance used by *P. contorta* for defense against this insect, as the tree and the insect species have their natural distributions in different parts of the world. However, phenylpropanoates may be of more general importance in plant–insect interactions than presently acknowledged, as indicated by the frequently strong antifeedant responses in the pine weevil.

Acknowledgments We thank The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning, The Swedish *Hylobius* Research Program, and The University of Kalmar for supporting this study. Henning Henschel, Abtin Daghigi, Meral Sari, Veronica Siljehav, Konstantin Dubrovinski, Tonja Freisolt, and Magnus Besev were helpful in the syntheses of the test compounds and Claes Hellqvist in processing the bioassay data.

References

BAECKSTRÖM, P., STRIDH, K., LI, L., and NORIN, T. 1987. Claisen rearrangements with mesityl oxide dimethyl ketal. Synthesis of ipsdienone, *E*- and *Z*-ocimenone, 2,6-dimethyl-2,7-octadien-4-one and 2,6-dimethyl-2,7-octadien-4-ol. *Acta Chem. Scand* B41:442–447.

BJÖRKLUND, N., NORDLANDER, G., and BYLUND, H. 2005. Olfactory and visual stimuli used in orientation to conifer seedlings by the pine weevil, *Hylobius abietis*. *Physiol. Entomol* 30:225–231.

BLANEY, W. M., SIMMONDS, M. S. J., EVANS, S. V., and FELLOWS, L. E. 1984. The role of the secondary plant compound 2,5-dihydroxymethyl 3,4-dihydroxypyrrolidine as a feeding inhibitor for insects. *Entomol. Exp. Appl* 36:209–216.

BORG-KARLSON, A.-K., NORDLANDER, G., MUDALIGE, A., NORDENHEM, H., and UNELIUS, C. R. 2006. Antifeedants in the feces of the pine weevil *Hylobius abietis*: Identification and biological activity. *J. Chem. Ecol* 32:943–957.

BRATT, K., SUNNERHEIM, K., NORDENHEM, H., NORDLANDER, G., and LÅNGSTRÖM, B. 2001. Pine weevil (*Hylobius abietis*) antifeedants from lodgepole pine (*Pinus contorta*). *J. Chem. Ecol* 27:2253–2262.

COWLES, R. S., MILLER, J. R., HOLLINGSWORTH, R. M., ABDEL-AAL, M. T., SZURDOKI, F., BAUER, K., and MATOLCSY, G. 1990. Cinnamyl derivatives and monoterpenoids as non-specific ovipositional deterrents for the onion fly. *J. Chem. Ecol* 16:2401–2428.

DAY, K. R., NORDLANDER, G., KENIS, M., and HALLDÓRSON, G. 2004. General biology and life cycles of bark weevils, Chapter 14, Bark and Wood Boring Insects in Living Trees in Europe, A Synthesis. pp. 331–349, in F. Lieutier, K. R. Day, A. Battisti, J. C Grégoire, and H. F. Evans (eds.). Kluwer, Dordrecht.

ERIKSSON, C. 2006, Isolation, synthesis and structure–activity relationships of antifeedants against the pine weevil, *Hylobius abietis*. Ph.D. Dissertation, KTH, Stockholm, Sweden. ISBN: 91-7178-301-6.

HANSCH, C., and FUJITA, T. 1963. ρ–σ–π Analysis. A method for the correlation of biological activity and chemical structure. *J. Am. Chem. Soc* 86:1616–1626.

HANSCH, C., and DEUTSCH, E. W. 1966. The structure–activity relationship in amides inhibiting photosynthesis. *Biochim. Biophys. Acta* 112:381–391.

HANSCH, C., LEO, A., UNGER, S. H., KI, H. K., NIKAITANI, D., and LIEN, E. J. 1973. "Aromatic" substituent constants for structure–activity correlations. *J. Med. Chem* 16:1207–1216.

HARWOOD, L. M., MOODY, C. J., and PERCY, J. M. 1998. Experimental Organic Chemistry. 2nd edn. Blackwell Science, Oxford.

HUANG, Y., and HO, S. H. 1998. Toxicity and antifeedant activities of cinnamaldehyde against the grain storage insects, *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch. *J. Stored Prod. Res* 34:11–17.

JACUBAS, W. J., SHAH, P. S., MASON, J. R., and NORMAN, D. M. 1992. Avian repellency of coniferyl and cinnamyl derivatives. *Ecol. Appl* 2:147–156.

LÅNGSTRÖM, B., and DAY, K. R. 2004. Damage, control and management of weevil pests, especially *Hylobius abietis*, Chapter 19, Bark and Wood Boring Insects in Living Trees in Europe, A Synthesis. pp. 415–444, in F. Lieutier, K. R. Day, A. Battisti, J. C Grégoire, and H. F. Evans (eds.). Kluwer, Dordrecht.

LEGRAND, S., NORDLANDER, G., NORDENHEM, H., BORG-KARLSON, A.-K., and UNELIUS, C. R. 2004. Hydroxy-methoxybenzoic methyl esters: synthesis and antifeedant activity on the pine weevil, *Hylobius abietis*. *Z. Naturforsch* 59b:829–835.

SUNNERHEIM, K., NORDQVIST, A., NORDLANDER, G., BORG-KARLSON, A.-K., UNELIUS, C. R., BOHMAN, B., NORDENHEM, H., HELLQVIST, C., and KARLÉN, A. 2007. Quantitative structure–activity relationships of pine weevil antifeedants, a multivariate approach. *J. Agric. Food Chem* 55:9365–9372.

SWAIN, C. G., and LUPTON, E. C. 1968. Field and resonance components of substituent effects. *J. Am. Chem. Soc* 90:4328–4337.

TOPLISS, J. G. 1972. Utilization of operational schemes for analog synthesis in drug design. *J. Med. Chem* 15:1006–1011.

TOPLISS, J. G. 1977. A manual method for applying the Hansch approach to drug design. *J. Med. Chem* 20:463–469.

UNELIUS, C. R., NORDLANDER, G., NORDENHEM, H., HELLQVIST, C., LEGRAND, S., and BORG-KARLSON, A.-K. 2006. Structure–activity relationships of benzoic acid derivatives as antifeedants for the pine weevil, *Hylobius abietis*. *J. Chem. Ecol* 32:2191–2203.

VOGEL, A. I. 1989. Textbook of Practical Organic Chemistry. 5th edn. Prentice Hall, Upper Saddle River, NJ.

WATKINS, R. W., LUMLEY, J. A., GILL, E. L., BISHOP, J. D., LANGTON, S. D., MACNICOLL, A. D., PRICE, N. R., and DREW, M. G. B. 1999. Quantitative structure–activity relationships (QSAR) of cinnamic acid bird repellents. *J. Chem. Ecol* 25:2825–2845.

WEISSENBERG, M., MEISNER, J., KLEIN, M., SCHAEFFLER, I., ELIYAHU, M., SCHMUTTERER, H., and ASCHER, K. R. S. 1997. Effect of substituent and ring changes in naturally occuring naphthoquinones on the feeding response of larvae of the mexican bean beetle, *Epilachna varivestis*. *J. Chem. Ecol* 23:3–18.

WIBE, A., BORG-KARLSON, A.-K., NORIN, T., and MUSTAPARTA, H. 1996. Identification of plant volatiles activating the same receptor neurons in the pine weevil, *Hylobius abietis*. *Entomol. Exp. Appl* 80:39–42.

WIBE, A., BORG-KARLSON, A.-K., NORIN, T., and MUSTAPARTA, H. 1997. Head-space plant volatiles eliciting responses in receptor neurons of the pine weevil (*Hylobius abietis* L.). *J. Comp. Physiol* 180:585–595.

WIBE, A., BORG-KARLSON, A.-K., PERSSON, M., NORIN, T., and MUSTAPARTA, H. 1998. Enantioselectivity of receptor neurons in the pine weevil (*Hylobius abietis*). *J. Chem. Ecol* 24:273–287.