Hydroxy-Methoxybenzoic Methyl Esters: Synthesis and Antifeedant Activity on the Pine Weevil, *Hylobius abietis*

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The pine weevil *Hylobius abietis* (L.) (Coleoptera: Curculionidae) feeds on the bark of coniferous seedlings and is the economically most important forestry restocking pest in large parts of Europe. Substances with an antifeedant effect may offer an environmentally friendly alternative to insecticides for the protection of planted seedlings. Bioassays were performed on commercial and synthetic methyl hydroxy-methoxybenzoates in order to determine their possible antifeedant activity.

Two methyl hydroxy-methoxybenzoates were synthesized by esterification and mono-*O*-methylation of two dihydroxybenzoic acids. A regioselective protection-deprotection strategy was used in the synthetic pathway of the other non-commercial esters, esterification and selective pivaloylation of the less-hindered hydroxyl group of other commercial dihydroxybenzoic acids gave diester intermediates, which then were *O*-methylated before methanolysis of the pivaloyl group which yielded the desired non-commercial methyl hydroxy-methoxybenzoates.

The five synthesized methyl hydroxy-methoxybenzoic esters were complemented with commercial samples of the five other isomers of methyl hydroxy-methoxybenzoate and spectrometric data were collected for the complete set of isomers. All ten isomers were tested for their antifeedant effect on the pine weevil. The effect varied considerably among the hydroxy-methoxybenzoic esters. Methyl 2-hydroxy-3-methoxybenzoate showed the highest effect and may thus be a candidate for practical use in pine weevil pest management.

Key words: Methyl Hydroxy-methoxybenzoates, Antifeedant Activity, *Hylobius abietis*

Introduction

Adult pine weevils, *Hylobius abietis* (L.), frequently kill planted conifer seedlings by their feeding on the stem bark. Unprotected seedlings commonly suffer over 80% mortality in regions with managed coniferous forests [1]. To protect the seedlings it is common practice in many European countries to routinely treat transplants with an insecticide. Because of environmental hazards and health risks for forest workers the usage of insecticides is seriously questioned today. Possibly, antifeedant substances applied to transplants could offer an alternative to insecticides [2].

Recently we have shown that various benzoate derivatives have strong antifeedant effect on the pine weevil [3]. This encouraged further studies of compounds related to benzoic acid. In this study, we investigated the potential of hydroxy-methoxy acid methyl esters as antifeedants useful for the protection of planted seedlings against pine weevil damage. There are 10 possible isomers of methyl hydroxy–methoxybenzoate (Scheme 1). The esters **1 – 5** had to be synthesized while the esters **6 – 10** were commercially available.

The esters **1, 2, 3** and **4** are intermediates in the total synthesis of compounds with important biological effects and their synthesis have been reported previously [4]. The synthesis of methyl 5-hydroxy-2-methoxybenzoate (**5**) was published in 1983 by Harwood [5].

The methyl benzoic esters **1** and **2** were synthesized based on the method described by Chakraborty

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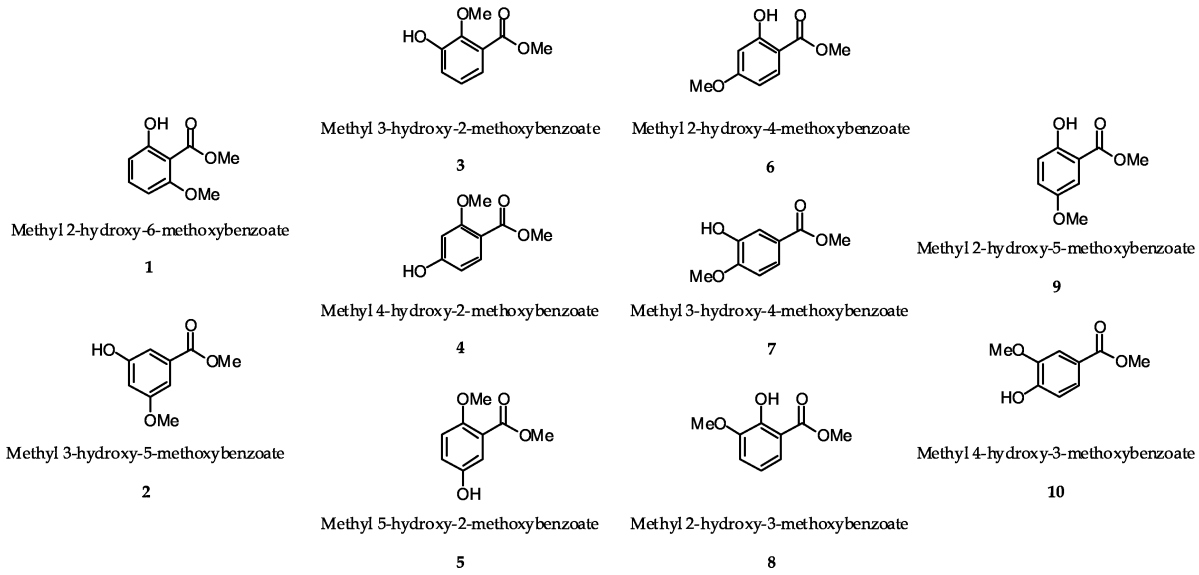
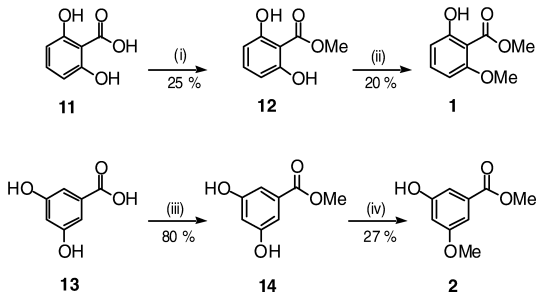


Scheme 1. All isomers of methyl hydroxy-methoxybenzoate (**1 – 10**).

et al. [4b]. The corresponding acids of **1** and **2** were esterified and mono-*O*-methylated (Scheme 2). The regioselective protection presented by Dornhagen and Scharf in their synthesis of the dichloroisoeverninic acid [6] was used as a basis for our synthesis of the methyl benzoates **3**, **4** and **5** (Scheme 3). Our synthesis started by esterification of the benzoic acids **15a**, **15b** and **15c**. Acylation of the synthesized esters **16a**, **16b** and **16c** occurred only at the less-hindered hydroxyl group (OH group *meta* or *para* to the ester group). *O*-methylation of the *ortho*-OH group, followed by deprotection of the diesters gave the desired methyl benzoic esters **3**, **4** and **5**.



Scheme 2. Reaction conditions: (i) DCC, MeOH, DMAP, CH_2Cl_2, RT; (ii) MeI, K_2CO_3, DMF, 35 °C; (iii) MeOH, H_2SO_4, reflux; (iv) MeI, MeOH, K_2CO_3, RT.

Results and Discussion

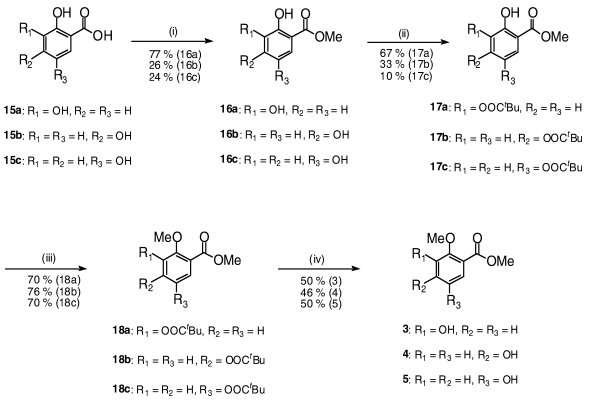
Methyl 2-hydroxy-6-methoxybenzoate (**1**) and methyl 3-hydroxy-5-methoxybenzoate (**2**) were synthesized from the commercially available benzoic acids **11** and **13** (Scheme 2). It was found that the esterification of the carboxylic acid **11** with MeOH and H_2SO_4 as reactants gave the ester **12** in very low yield. The low reactivity of the COOH group in **11** is presumably due to the resonance effect of two hydroxyl groups *ortho* to COOH. The yield of this esterification reaction was improved when the compound **11** was treated with dicyclocarbodiimide (DCC) and dimethylaminopyridine (DMAP) in a MeOH/CH_2Cl_2 mixture. The esterification conditions were more effective in this case since the carboxylic acid was converted to a compound with a better leaving group. It was noted that the treatment of the *meta* disubstituted benzoic acid **13** with an excess of MeOH and a catalytic amount of H_2SO_4 afforded the ester **14** in good yield, due to the absence of resonance effects with the COOH group. The products **12** and **14** were then mono-*O*-methylated by use of methyl iodide in the presence of a weak base.

A regioselective protection [6] was the key step in the syntheses of the methyl benzoates **3**, **4** and **5** (Scheme 3). After esterification of the commercially available benzoic acids **15a**, **15b** or **15c**, it was found that the esters **16a**, **16b** or **16c** when reacted with trimethylacetyl chloride, selectively yielded the intermediates **17a**, **17b** or **17c**.

No	Compound	Index (6 h)	Level of significance	Index (24 h)	Level of significance
1	Methyl 2-hydroxy-6-methoxybenzoate	94	***	54	***
2	Methyl 3-hydroxy-5-methoxybenzoate	35	***	26	***
3	Methyl 3-hydroxy-2-methoxybenzoate	77	***	35	***
4	Methyl 4-hydroxy-2-methoxybenzoate	69	***	4	ns
5	Methyl 5-hydroxy-2-methoxybenzoate	4	ns	−3	ns
6	Methyl 2-hydroxy-4-methoxybenzoate	100	***	52	***
7	Methyl 3-hydroxy-4-methoxybenzoate	69	***	32	***
8	Methyl 2-hydroxy-3-methoxybenzoate	100	***	85	***
9	Methyl 2-hydroxy-5-methoxybenzoate	93	***	56	***
10	Methyl 4-hydroxy-3-methoxybenzoate	56	***	22	*

Table 1. Effect of the ten benzoates on bark feeding by the pine weevil *Hylobius abietis*, as measured by the antifeedant index (0 is no activity, 100 is complete feeding deterrence).

$* = p < 0.05$, $** = p < 0.01$, $*** = p < 0.001$ (Fisher exact test of a 2×2 table).

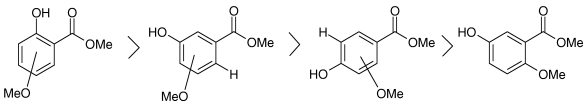


Scheme 3. Reaction conditions: (i) MeOH, H_2SO_4, reflux; (ii) trimethylacetyl chloride, pyridine, CH_2Cl_2, −10 °C to RT; (iii) MeI, K_2CO_3, DMF, 35 °C; (iv) MeOH, K_2CO_3, RT.

Due to the steric hindrance between the bulky protecting group, ^{t}Bu, and the ester moiety, acylation was predominant at hydroxyl groups *meta* and *para* to the carbomethoxy group and not with the hydroxyl group *ortho*. *O*–methylation of the hydroxyl group *ortho* to the carbomethoxy group gave the compounds **18a**, **18b** or **18c**. Then, the hydroxyl groups *meta* or *para* to the carbomethoxy group were deprotected using MeOH/K_2CO_3, yielding the desired methyl benzoates **3**, **4** or **5**.

In conclusion, the synthesis of all non-commercial methyl hydroxy-methoxybenzoate was presented. Starting from the benzoic esters **11** and **13**, the methyl esters **1** and **2** were synthesized in two steps. A regioselective protection was the critical step in the syntheses of the other methyl benzoates **3**, **4** and **5**.

The spectroscopic data of the commercially available methyl benzoates **6**, **7**, **8**, **9** and **10** were also recorded. Interestingly, we noted that the mass spectra of all 2-hydroxy-isomers have a strong *m/z* 150 *i.e.* loss of methanol (32), while all other isomers have a strong 151 fragment. The mechanism for the loss



Scheme 4. Structure – activity relationships (decreasing activity from left to right).

of methanol can be explained by a rearrangement between the methylcarboxylate moiety and a hydroxyl hydrogen in ortho-position [7].

Bioassays were performed with all esters in order to determine their possible antifeedant effect against the pine weevil. Eight of the ten compounds showed antifeedant activity after 24 h exposure to pine weevils in the bioassay (Table 1). Only compounds **4** and **5** did not inhibit feeding over the 24 h period, although **4** showed activity after 6 h. The most potent antifeedant among these compounds was **8**. It was closely followed in activity by compounds **9**, **1**, and **6**, and thereafter **3** and **7**. Compound **2** and, particularly, **10** had only a weak effect.

Apparently, isomers with a hydroxy group in the ortho position have a stronger antifeedant effect (Scheme 4). The most potent compound (**8**) gave a somewhat higher index value after 24 h than shown by the strongest antifeedant compound (ethyl cinnamate) recently isolated from bark of *Pinus contorta* [2].

Conclusion

Starting from commercially available hydroxy-methoxybenzoic acids, all non-commercial methyl hydroxy-methoxybenzoates were synthesized. Among the methyl hydroxy-methoxybenzoic esters tested in the bioassay, methyl 2-hydroxy-3-methoxybenzoate had the strongest antifeedant effect on adult pine weevils. A comparison with previously discovered antifeedants indicates that methyl 2-hydroxy-3-methoxybenzoate has potential for use in practical protection of conifer transplants. Further synthesis and bioassays

are needed to predict the optimal structure for maximal antifeedant activity. More comparisons of similar compounds are also needed before structure – activity patterns can be properly discussed.

Experimental Section

Synthesis: General synthetic methodology

Melting points were determined on a Büchi 510 instrument and were not corrected. Preparative chromatography [8] and flash chromatography were done on silica gel (Merck 60). NMR spectra were recorded on spectrometers Bruker AC 250 (250 MHz for ^{1}H and 63 MHz for ^{13}C) and Bruker AMX 500 (500 MHz for ^{1}H and 125 MHz for ^{13}C). $CDCl_3$ and DMSO-d_6 were used as solvents and the signals of the solvents served as internal standards. Chemical shifts were expressed in ppm, followed by multiplicity (s, singlet; t, triplet; d, doublet; m, multiplet; b, broad) and number of protons. Mass spectra of positive ions obtained by electron impact (EI, 70 eV) were measured on Hewlett-Packard or Varian Saturn ws GC-MS instruments.

Dimethylformamide (DMF) was distilled under N_2 before use. Pyridine and CH_2Cl_2 were dried over 4 Å molecular sieves. The starting materials employed were purchased from commercial suppliers and were used without further purification.

Methyl 2,6-dihydroxybenzoate (**12**): 2,6-Dihydroxybenzoic acid (**11**) (1.00 g, 6.49 mmol) was dissolved in MeOH (10 ml) and CH_2Cl_2 (65 ml) was added to the reaction mixture. DCC (1,3-dicyclohexylcarbodiimide) (1.49 g, 7.14 mmol) and DMAP (4-dimethylaminopyridine) (0.158 g, 1.30 mmol) were added and the reaction mixture was stirred at room temperature (RT) for 72 h. The white precipitate was then removed by filtration and the solvents were evaporated. The crude product was purified by flash chromatography on silica gel, using cyclohexane–EtOAc (3:2) as eluent, to give **12** (277 mg, 25%) as a white solid. M.p. 58 – 60 °C. – ^{1}H NMR (250 MHz, DMSO–d_6): $\delta = 3.78$ (s, 3 H, COOMe), 6.32 – 6.35 (d, 2 H, 2×H_{ar}) 7.05 – 7.12 (t, 1 H, H_{ar}), 9.94 (bs, 2 H, OH). – ^{13}C{^{1}H} NMR (62.9 MHz, DMSO–d_6): $\delta = 51.76$ (COO*Me*), 106.60 (2×C_{ar}), 106.88, 132.27, 157.22, 157.24, 168.17 (all C_{ar} and C=O). – MS: $m/z = 168$ [M^+], 153, 136 (100%), 108, 96, 80, 69, 63, 52, 44, 39.

Methyl 2-hydroxy-6-methoxybenzoate (**1**): Methyl 2,6-dihydroxybenzoate (**12**) (260 mg, 1.55 mmol) was dissolved in DMF (2 ml) and K_2CO_3 (256 mg, 1.86 mmol) was added in 2 portions, followed by MeI (0.12 ml, 1.94 mmol). The resulting suspension was vigorously stirred at 35 °C for 3 h. The reaction mixture was then cooled to room temperature, the solid was removed by filtration and the solvent was evaporated to give a brown oil. The crude oil was purified by two flash chromatography procedures using cyclohexane–ethyl acetate (EtOAc) (2:3) and cyclohexane–EtOAc (4:1) as eluents. Compound **1** was isolated as a white solid (55 mg, 20%). M.p. 50 °C. – ^{1}H NMR (250 MHz, DMSO–d_6): $\delta = 3.71$ (s, 3 H, OMe), 3.73 (s, 3 H, COOMe), 6.47 – 6.51 (d, 2 H, 2×H_{ar}), 7.14 – 7.21 (t, 1 H, H_{ar}), 9.98 (bs, 1 H, OH). – ^{13}C{^{1}H} NMR (62.9 MHz, DMSO–d_6): $\delta = 51.64$ (COO*Me*), 55.54 (O*Me*) 101.84, 106.59, 108.38, 130.87, 155.32, 157.03, 166.52 (all C_{ar} and C=O). – MS: m/z(%) = 182 (38) [M^+], 150 (100), 136 (5.7), 122 (31), 107 (55), 93 (2.9), 79 (5.3), 65 (5.3), 51 (4.3), 39 (4.3).

Methyl 3,5-dihydroxybenzoate (**14**): 3,5-Dihydroxybenzoic acid (**13**) (1.00 g, 6.49 mmol) was dissolved in MeOH (40 ml) and some drops of H_2SO_4 were slowly added to the reaction mixture, which was stirred at reflux temperature. The reaction was monitored by TLC. When the reaction was finished, the solvent was evaporated and the crude product was dissolved in EtOAc and washed twice with brine. The organic layer was dried over $MgSO_4$ and the solvent was evaporated to give **14** as a white powder (871 mg, 80%). M.p. 165 – 168 °C. – ^{1}H NMR (250 MHz, DMSO–d_6): $\delta = 3.78$ (s, 3 H, COOMe), 6.43 (m, 1 H, H_{ar}), 6.80 (m, 2 H, 2×H_{ar}), 9.64 (s, 2 H, OH). – ^{13}C{^{1}H} NMR (62.9 MHz, DMSO–d_6): $\delta = 51.85$ (COO*Me*), 106.95 (2×C_{ar}), 107.04, 131.16 (all C_{ar}), 158.41 (2×C_{ar}), 166.12 (C=O). – MS: $m/z = 168$ [M^+], 137 (100%), 109, 95, 81, 69, 53, 44.

Methyl 3-hydroxy-5-methoxybenzoate (**2**): Methyl 3,5-dihydroxybenzoate (**14**) (300 mg, 1.78 mmol) was dissolved in MeOH, K_2CO_3 (296 mg, 2.14 mmol) was added and the reaction mixture was stirred for a couple of minutes. MeI (0.11 ml, 1.78 mmol) was then added and the mixture was stirred at room temperature overnight. Silica gel was then added and the solvent was evaporated. After drying, the impregnated silica gel was put on top of a chromatography column and subjected to medium pressure liquid chromatography (MPLC, cyclohexane:EtOAc 70:30) to give **2** as a white powder (70 mg, 21%). M.p. 82 – 84 °C. – ^{1}H NMR (250 MHz, DMSO–d_6): $\delta = 3.75$ (s, 3 H, COOMe), 3.82 (s, 3 H, OMe), 6.58 (s, 1 H, H_{ar}), 6.91 (s, 1 H, H_{ar}), 6.97 (s, 1 H, H_{ar}), 9.87 (s, 1 H, OH). – ^{13}C{^{1}H} NMR (62.9 MHz, DMSO–d_6): $\delta = 52.04$ (COO*Me*), 55.15 (OMe), 104.99, 105.99, 108.54, 131.35, 158.53, 160.35, 165.95 (all C_{ar} and C=O). – MS: m/z(%) = 182 (93) [M^+], 167 (1), 151 (100), 136 (2.9), 123 (34), 108 (22), 93 (8.6), 79 (3.3), 69 (16), 63 (4.8), 51 (4.8), 44 (9), 39 (3.8).

Methyl 2,3-dihydroxybenzoate (**16a**): Prepared by the procedure used for compound **14** but with 2,3-dihydroxybenzoic acid (**15a**) (1.50 g, 9.8 mmol) as starting material. **16a** was isolated as a slightly brown solid (1.27 g, 77%). M.p. 68 – 71 °C. – ^{1}H NMR (250 MHz, DMSO–d_6): $\delta = 3.88$ (s, 3 H, COOMe), 6.75 (m, 1 H, H_{ar}), 7.01 (m, 1 H, H_{ar}), 7.22 (m, 1 H, H_{ar}), 9.44 (s, 1 H, OH), 10.41 (s, 1 H, OH). – ^{13}C{^{1}H} NMR (62.9 MHz, DMSO–d_6): $\delta = 52.30$ (COO*Me*), 112.96, 118.81, 119.42, 120.58, 145.97, 149.26, 169.71 (all C_{ar} and

C=O). MS: $m/z = 168$ [M^+], 153, 136 (100%), 119, 108, 91, 80, 63, 52, 44, 39.

Methyl 2,4-dihydroxybenzoate (**16b**): Prepared by the same procedure as compound **14** but with 2,4-dihydroxybenzoic acid **15b** (5.00 g, 32.44 mmol) as starting material. The crude product was purified by flash chromatography on silica gel using cyclohexane–EtOAc (80:20) as eluent. A white solid **16b** (1.43 g, 26%) was obtained. M.p. 115–118 °C. – ^{1}H NMR (250 MHz, DMSO–d_6): $\delta = 3.83$ (s, 3 H, COOMe), 6.29–6.30 (d, 1 H, H_{ar}), 6.34–6.38 (dd, 1 H, H_{ar}), 7.61–7.65 (d, 1 H, H_{ar}), 10.46 (s, 1 H, OH), 10.71 (s, 1 H, OH). – ^{13}C{^{1}H} NMR (62.9 MHz, DMSO–d_6): $\delta = 51.90$ (COO*Me*), 102.36, 103.86, 108.24, 131.50, 162.58, 164.11, 169.46 (all C_{ar} and C=O). – MS: $m/z = 168$ [M^+], 136 (100%), 125, 108, 95, 80, 69, 63, 53, 44, 39.

Methyl 2,5-dihydroxybenzoate (**16c**): Prepared by the procedure used for compound **14** but with 2,5-dihydroxybenzoic acid **15c** (3.00 g, 19.4 mmol) as starting material. **16c** was isolated as a white solid (0.78 g, 24%). M.p. 73–76 °C. – ^{1}H NMR (250 MHz, DMSO–d_6): $\delta = 3.87$ (s, 3 H, COOMe), 6.76–6.83 (m, 1 H, H_{ar}), 6.93–6.99 (m, 1 H, H_{ar}), 7.14 (m, 1 H, H_{ar}), 9.18 (s (apparent d), 1 H, OH), 9.85 (bs, 1 H, OH). – ^{13}C{^{1}H} NMR (62.9 MHz, DMSO–d_6): $\delta = 52.32$ (COO*Me*), 114.01, 117.63, 123.74, 149.47, 153.02, 153.97, 171.61 (all C_{ar} and C=O). – MS: $m/z = 168$ [M^+], 136, 108, 80, 69, 53, 44.

Methyl 2-hydroxy-3-pivaloyloxybenzoate (**17a**): Methyl benzoate **16a** (800 mg, 4.76 mmol) was dissolved in CH_2Cl_2 (8.4 ml) under inert atmosphere and pyridine (2.6 ml) was added to the reaction mixture. The reaction mixture was then cooled to −10 °C and a solution of pivaloyl chloride (642 mg, 5.30 mmol) in CH_2Cl_2 (0.7 ml) was added drop wise to the reaction mixture, which was allowed to reach RT. After stirring for 48 h, the solvent was evaporated and the crude crystals were purified by two consecutive flash chromatography treatments using cyclohexane-EtOAc (7:3) and cyclohexane-Et_2O (6:1) as eluents. This procedure yielded **17a** as a white solid (803 mg, 67%). M.p. 64–67 °C. – ^{1}H NMR (250 MHz, DMSO–d_6): $\delta = 1.31$ (s, 9 H, 3×Me), 3.77–3.91 (s (app. d), 3 H, COOMe), 6.97–7.71 (m, 3 H, 3×H_{ar}), 10.52 (bs, 1 H, OH). – ^{13}C{^{1}H} NMR (62.9 MHz, DMSO–d_6): $\delta = 26.74$ (4×Me), 38.38 (COO*Me*), 52.61 (C_q), 118.83, 120.49, 125.86, 126.97, 128.62, 139.06, 152.10, 168.96 (all C_{ar} and 2×C=O), 168.96 (C=O). – MS: $m/z = 252$ [M^+], 168, 136 (100%), 107, 85, 69, 57, 41.

Methyl 2-hydroxy-4-pivaloyloxybenzoate (**17b**): Prepared by the procedure used for compound **17a** but with the methyl benzoate **15b** (500 mg, 2.97 mmol) as starting material. Compound **17b** was isolated as a white solid (245 mg, 33%). M.p. 71–73 °C. – ^{1}H NMR (250 MHz, DMSO–d_6): $\delta = 1.30$ (s, 9 H, 3×Me), 3.89 (s, 3 H, COOMe), 6.69–6.77 (t, 2 H, 2×H_{ar}), 7.80–7.85 (d, 1 H, H_{ar}). – ^{13}C{^{1}H} NMR (62.9 MHz, DMSO–d_6): $\delta = 26.50$ (4×Me), 38.52 (COO*Me*), 52.32 (C_q), 110.32, 110.83, 113.20, 131.19, 155.91, 160.93, 168.36, 175.57 (all C_{ar} and 2×C=O). – MS: $m/z = 252$ [M^+], 221, 168, 136 (100%), 108, 95, 85, 69, 57, 41.

Methyl 2-hydroxy-5-pivaloyloxybenzoate (**17c**): Prepared by the procedure used for compound **17a** but with the methyl benzoate **16c** (650 mg, 3.87 mmol) as starting material. Compound **17c** was isolated as a white solid (98 mg, 10%). – ^{1}H NMR (250 MHz, DMSO–d_6): $\delta = 1.28$ (s, 9 H, 3×Me), 3.88 (s, 3 H, COO*Me*), 6.99–7.03 (d, 1 H, H_{ar}), 7.25–7.29 (m, 1 H, H_{ar}), 7.29–7.43 (m, 1 H, H_{ar}), 10.50 (bs, 1 H, OH). – MS: $m/z = 252$ [M^+], 221, 205, 193, 177, 168, 136 (100%), 108, 85, 77, 69, 57, 50, 41.

Methyl 2-methoxy-3-pivaloyloxybenzoate (**18a**): The diester **17a** (400 mg, 1.59 mmol) was dissolved in dry DMF (2 ml) and K_2CO_3 (242 mg, 1.90 mmol) was added in 2 portions, followed by MeI (0.128 ml, 2.06 mmol). The resulting suspension was stirred vigorously at 35 °C for 90 min. The reaction mixture was then cooled to RT. The solid was removed by filtration and the solvent was evaporated to give an oil. The solid was dissolved in water (10 ml) and added to the oil. The water phase was extracted with Et_2O (3×10 ml). The combined organic layers were washed with water and brine. The organic layer was then dried over $MgSO_4$ and the solvent was evaporated to give **18a** as a pale yellow oil (288 mg, 70%). – ^{1}H NMR (250 MHz, DMSO–d_6): $\delta = 1.31 - 1.33$ (s (app. d), 9 H, 3×CH_3), 3.72 (s, 3 H, OMe), 3.85 (s, 3 H, COOMe), 7.21–7.39 (m, 2 H, 2×H_{ar}),7.60–7.63 (m, 1 H, H_{ar}). – ^{13}C{^{1}H} NMR (62.9 MHz, DMSO–d_6): $\delta = 26.62$ (4×Me), 38.37 (COO*Me*), 52.19 (C_q), 61.95 (OMe), 124.02, 125.83, 127.35, 127.95, 144.54, 151.01, 165.21, 175.70 (all C_{ar} and 2×C=O). – MS: $m/z = 266$ [M^+], 235, 219, 182, 164, 150, 136, 121, 107, 93, 85, 77, 65, 57 (100%), 41.

Methyl 2-methoxy-4-pivaloyloxybenzoate (**18b**): Produced by the procedure employed for compound **18a** but with the diester **17b** (200 mg, 0.793 mmol) as starting material. Compound **18b** was isolated as a colourless oil (160 mg, 76%). – ^{1}H NMR (250 MHz, DMSO–d_6): $\delta = 1.30$ (s, 9 H, 3×Me), 3.78 (s, 3 H, COOMe), 3.82 (s, 3 H, OMe), 6.74–6.78 (m, 1 H, H_{ar}), 6.91–6.92 (m, 1 H, H_{ar}), 7.70–7.73 (m, 1 H, H_{ar}). – ^{13}C{^{1}H} NMR (62.9 MHz, DMSO–d_6): $\delta = 26.56$ (4×Me), 38.52 (COO*Me*), 51.72 (C_q), 56.06 (OMe), 106.45, 113.35, 117.16, 131.70, 154.65, 159.35, 165.31, 175.71 (all C_{ar} and 2×C=O). – MS: $m/z = 266$ [M^+], 235, 223, 182, 165, 151, 136, 122, 107, 93, 85, 77, 65, 57 (100%), 41.

Methyl 2-methoxy-5-pivaloyloxybenzoate (**18c**): Synthesized by the procedure used for compound **18a** but with the diester **17c** (100 mg, 0.39 mmol) as starting material. Compound **18c** was isolated as a colourless oil (74 mg, 70%). – ^{1}H NMR (250 MHz, DMSO–d_6): $\delta = 1.28$ (s, 9 H, 3×Me), 3.78 (s, 3 H, COOMe), 3.82 (s, 3 H, OMe), 7.16–7.36 (m, 3 H, 3×H_{ar}). – ^{13}C{^{1}H} NMR (62.9 MHz, DMSO–d_6): $\delta =$ 26.65 (4×Me), 38.52 (COO*Me*), 51.97 (C_q), 56.14 (OMe),

113.42, 120.28, 123.45, 126.43, 143.12, 155.66, 165.17, 176.45 (all C_{ar} and 2×C=O). – MS: $m/z = 266$ [M^+], 235, 182 (100%), 167, 149, 135, 121, 107, 93, 85, 77, 65, 57, 41.

Methyl 3-hydroxy-2-methoxybenzoate (**3**): The diester **18a** (98 mg, 0.37 mmol) was dissolved in MeOH (3.7 ml) and K_2CO_3 (0.108 g, 0.78 mmol) was added to the reaction mixture that was stirred at room temperature for 3 h. Then the liquid was decanted from the solid residue and the solvent was evaporated to give crude white crystals. The previous solid residue was dissolved in water (3 ml) and HCl (37%) was added until pH=2. Then the aqueous solution was added to the crude white crystals and the mixture was extracted with Et_2O (3×5 ml). The combined organic layers were washed (water, brine) and dried ($MgSO_4$). The solvent was evaporated to give **3** as a colourless oil (34 mg, 50%). – ^{1}H NMR (250 MHz, DMSO–d_6): $\delta = 3.74$ (s, 3 H, COOMe), 3.80 (s, 3 H, OMe), 7.03 (m, 3 H, 3×H_{ar}), 9.64 (bs, 1 H, OH). – ^{13}C{^{1}H} NMR (62.9 MHz, DMSO–d_6): $\delta = 51.82$ (COO*Me*), 60.51 (OMe), 119.87, 119.97, 123.79, 125.80, 146.62, 150.83, 166.20 (all C_{ar} and C=O). – MS: $m/z(\%) = 182$ (81) [M^+], 164 (24), 151 (80), 136 (43), 121 (100), 107 (62), 93 (12), 79 (12), 65 (16), 59 (2.4), 51 (14), 45 (3.8), 39 (6.7).

Methyl 4-hydroxy-2-methoxybenzoate (**4**): Prepared by the procedure used for compound **3** with the diester **18b** (160 mg, 0.60 mmol) as starting material. Compound **4** was isolated as a white solid (51 mg, 46%). M.p. 130–135 °C. – ^{1}H NMR (250 MHz, DMSO–d_6): $\delta = 3.70$ (s, 3 H, COOMe), 3.75 (s, 3 H, OMe), 6.38–6.46 (m, 2 H, 2×H_{ar}), 7.58–7.62 (m, 1 H, H_{ar}), 10.36 (bs, 1 H, OH). – ^{13}C{^{1}H} NMR (62.9 MHz, DMSO–d_6): $\delta = 51.10$ (COO*Me*), 55.38 (OMe), 99.45, 107.10, 109.85, 133.14, 160.84, 162.62, 165.31 (all C_{ar} and C=O). – MS: $m/z(\%) = 182$ (32) [M^+], 151 (100), 136 (5.2), 121 (12), 108 (12), 93 (5.7), 65 (5.7), 53 (5.3), 44 (0.4), 39 (5.7).

Methyl 5-hydroxy-2-methoxybenzoate (**5**): Prepared by the procedure used for compound **3** but with the diester **18c** (288 mg, 1.08 mmol) as starting material. Purification of the crude product by flash chromatography on silica gel using cyclohexane–EtOAc (3:1) as eluent yielded **5** as a slightly yellow oil (93 mg, 50%). – ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 3.83$ (s (app. d), 3 H, COOMe), 3.88 (s (app. d), 3 H, OMe), 6.83–6.98 (m, 2 H, 2×H_{ar}), 7.24–7.34 (m, 1 H, H_{ar}). – ^{13}C{^{1}H} NMR (62.9 MHz, $CDCl_3$): $\delta = 52.25$ (COO*Me*), 56.63 (OMe), 113.89, 118.12, 120.71, 122.48, 149.22, 153.35, 166.86 (all C_{ar} and C=O). – MS: $m/z(\%) = 182$ (75) [M^+], 167 (6.7), 151 (100), 136 (17), 121 (15), 108 (21), 93 (20), 80 (9), 65 (18), 52 (15), 44 (5.7).

Characterization of commercially available isomers, all purchased from Aldrich.

Methyl 2-hydroxy-4-methoxybenzoate (**6**): M.p. 50–53 °C. – ^{1}H NMR (500.14 MHz, DMSO–d_6): $\delta = 3.81$ (s, 3 H, COOMe), 3.87 (s, 3 H, OMe), 6.52–6.54 (m, 2 H, 2×H_{ar}), 7.71–7.73 (d, 1 H, H_{ar}), 10.78 (bs, 1 H, OH). – ^{13}C{^{1}H} NMR (125.76 MHz, DMSO–d_6): $\delta = 53.06$ (COO*Me*), 56.49 (OMe), 101.84, 106.15, 108.32, 132.21, 163.49, 166.11, 170.25 (all C_{ar} and C=O). – MS: $m/z(\%) = 182$ (40) [M^+], 168 (2), 150 (100), 139 (3.5), 122 (57), 107 (28), 95 (10), 79 (18), 63 (5), 51 (7.5).

Methyl 3-hydroxy-4-methoxybenzoate (**7**): M.p. 64–67 °C. – ^{1}H NMR (500.14 MHz, DMSO–d_6): $\delta = 3.79$ (s, 3 H, COOMe), 3.84 (s, 3 H, OMe), 7.01–7.04 (d, 1 H, H_{ar}), 7.37–7.40 (d, 1 H, H_{ar}), 7.43–7.47 (dd, 1 H, H_{ar}), 9.48 (bs, 1 H, OH). – ^{13}C{^{1}H} NMR (125.76 MHz, DMSO–d_6): $\delta = 52.62$ (COO*Me*), 56.47 (OMe), 112.28, 116.56, 122.35, 122.74, 147.13, 152.78, 166.92 (all C_{ar} and C=O). – MS: $m/z(\%) = 182$ (54) [M^+], 167 (5), 151 (100), 139 (4), 123 (13), 108 (7.5), 95 (2), 79 (6), 65 (6), 51 (7), 39 (2.5).

Methyl 2-hydroxy-3-methoxybenzoate (**8**): M.p. 61.5–62.5 °C. – ^{1}H NMR (500.14 MHz, DMSO–d_6): $\delta = 3.81$ (s, 3 H, COOMe), 3.90 (s, 3 H, OMe), 6.88–6.90 (t, 1 H, H_{ar}), 7.22–7.24 (d, 1 H, H_{ar}), 7.35–7.36 (d, 1 H, H_{ar}), 10.50 (bs, 1 H, OH). – ^{13}C{^{1}H} NMR (125.76 MHz, DMSO–d_6): $\delta = 53.40$ (COO*Me*), 56.78 (OMe), 113.89, 117.89, 119.69, 121.63, 149.13, 151.37, 170.50 (all C_{ar} and C=O). – MS: $m/z(\%) = 182$ (58) [M^+], 167 (2), 150 (65), 136 (7), 122 (100), 107 (28), 92 (18), 79 (13), 65 (9), 53 (11), 39 (5).

Methyl 2-hydroxy-5-methoxybenzoate (**9**): B.p. 235–240 °C. – ^{1}H NMR (500.14 MHz, DMSO–d_6): $\delta = 3.72$ (s, 3 H, COOMe), 3.89 (s, 3 H, OMe), 6.91–6.93 (dd, 1 H, H_{ar}), 7.13–7.16 (dd, 1 H, H_{ar}), 7.21–7.22 (d, 1 H, H_{ar}), 10.09 (bs, 1 H, OH). – ^{13}C{^{1}H} NMR (125.76 MHz, DMSO–d_6): $\delta = 53.30$ (COO*Me*), 56.41 (OMe), 112.97, 113.43, 119.36, 124.26, 152.56, 155.23, 169.90 (all C_{ar} and C=O). – MS: $m/z(\%) = 182$ (43) [M^+], 167 (2.5), 150 (100), 135 (15), 122 (20), 107 (30), 93 (7.5), 79 (27), 65 (5), 51 (10), 39 (2.5).

Methyl 4-hydroxy-3-methoxybenzoate (**10**): M.p. 69–70 °C. – ^{1}H NMR (500.14 MHz, DMSO–d_6): $\delta = 3.80$ (s, 3 H, COOMe), 3.82 (s, 3 H, OMe), 6.86–6.88 (d, 1 H, H_{ar}), 7.44–7.45 (d, 1 H, H_{ar}), 7.46–7.48 (dd, 1 H, H_{ar}), 9.96 (bs, 1 H, OH). – ^{13}C{^{1}H} NMR (125.76 MHz, DMSO–d_6): $\delta = 52.58$ (COO*Me*), 56.47 (OMe), 113.34, 116.06, 121.31, 124.28, 148.22, 152.38, 166.93 (all C_{ar} and C=O). – MS: $m/z(\%) = 182$ (55) [M^+], 167 (5), 151 (100), 140 (5), 124 (11), 108 (6), 93 (2), 79 (5), 65 (5), 51 (6), 39 (2.5).

Bioassay

The various esters were tested for antifeedant effect on the pine weevil *Hylobius abietis* (L.) (Coleoptera, Curculionidae). For each test, 40 pine weevils (20 females + 20 males) were used. They were placed in separate Petri dishes provided with a pine twig prepared with delimited treatment and control areas. These pine twigs were enveloped in aluminium 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 aluminium foil inside the rings, one of the two surfaces exposed was treated with 100 μl of a 50 mM methanol solution of the compound that was tested, and the other surface was treated with the same amount of methanol alone (control). The following day, after the solvent had evaporated, the metal rings were removed and the test started. After 6 and 24 hours it was recorded whether the pine weevil had started to feed on the treated and untreated surfaces. The antifeedant effect was expressed by means of the following index: (C-T)$\times$100/(C+T), wherein C is the number of control surfaces with feeding marks and T is the number of treated surfaces with feeding marks. It was tested if there was a statistic significant difference between treatment and control with a Fisher exact test of a 2×2 table.

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