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Structure-activity Relationship for the Insect Antifeedant Activity of Benzofuran Derivatives

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Coumaran (2,3-dihydrobenzofuran), a secondary metabolite of *Cyperus nipponicus*, inhibits the feeding of polyphagous insects. This secondary metabolite is regarded as one of the defensive systems of the Cyperaceae. A number of naturally occurring benzofurans that differ in their substitution pattern and oxidation state have been investigated for their ability to inhibit insect feeding by a bioassay with the common cutworm (*Spodoptera litura* F. Noctuidae) that applies the leaf disk method. The evaluation of the antifeedant activity of each test compound used the ED_{50} value based on the dose-response curve that was calculated with the probit method. The 2,3-dihydrobenzofuran derivative, 7-acetyl-4,6-dimethoxy-2-isopropenyl-2,3-dihydrobenzofuran, had an ED_{50} value of 1.3 μg (5.4×10^{-9} mol)/cm^2 against the common cutworm. The introduction of methoxy and acetyl groups increased the insect antifeedant activity. Furthermore, the insect antifeedant activity increased with decreasing lipophilicity of the test compounds.

Key words: insect antifeedant; 2,3-dihydrobenzofuran; structure-activity relationship; *Spodoptera litura*

A number of natural products that are sources of pest control agents are also lead compounds for the development of novel agrochemicals. Repellents are more biofriendly than exterminating agents. Insect antifeedants act as repellents and often have only weak insecticidal activity. One group of plants indigenous to temperate regions possessing insect antifeedant activity is the genus *Cyperus*. The major chemical constituents of these plants are terpenoid, quinone and benzofuran.[1,2] A number of compounds produced by this genus have shown a growth inhibitory effect against insects; *e.g.*, insect juvenile hormone III in rice flatsedge *Cyperus iria*[3] and insecticidal α-cyperone in purple nutsedge *Cyperus rotundus*.[4] Insect herbivores tend not to attack *Cyperus* species in their natural habitat, with the notable exception of larvae of the specialist moth, *Calamotropha shichito*. We have previously investigated extracts of Cyperaceae for potential insect antifeedant activity, and the active compound is known to be remirol (5-acetyl-4-hydroxy-2-isopropenyl-6-methoxy-2,3-dihydrobenzofuran).[5]

A study of the construction of the 2,3-dihydrobenzofuran moiety by condensation of phenol and alkylbromide has been conducted by Yamaguchi and co-workers (scheme).[6] However, it is also important to find analogues with higher activity than that of the natural product and to elucidate the structural requirements for agents which inhibit feeding. After examining the insect antifeedant activities of many of the benzofurans, they concluded that hydroxyl derivatives decreased the insect antifeedant activity.

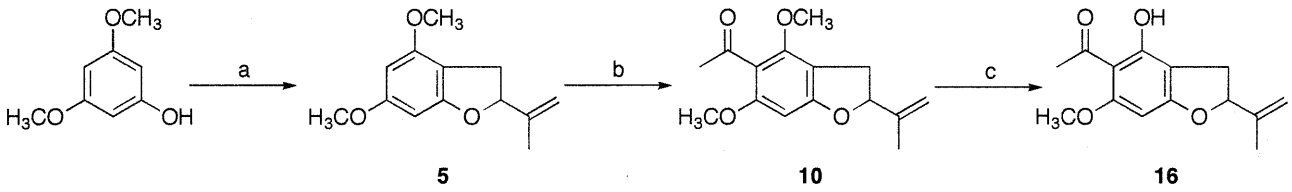
In this study, we evaluate the benzofuran derivatives as insect antifeedants against the common cutworm (*S. litura*) and report the use of the synthetic coumarans (2,3-dihydrobenzofuran) as an insect antifeedant against *S. litura*.

Materials and Methods

Apparatus. Melting point (mp) data were measured with Yanako MP apparatus. Mass spectra were obtained with a Shimadzu 9100MK GC-MS spectrometer at 70 eV, and NMR spectra were obtained with JEOL JNM-EX270 apparatus. TLC was performed with Merck silica gel 60 F_{254} plates 0.25 mm thick. Silica gel (Fuji Silysia Chemical BW-127ZH, BW-300) was used for column chromatography.

Isolation of remirol (**1**). Roots and basal stems of *Cyperus nipponicus* were collected in Osaka Pref. They were extracted with hexane and concentrated under reduced pressure. The resulting extract was chromatographed on silica gel, using hexane-ethyl acetate (10:1) as the eluent, to give natural product **1**, mp 76–77°C, as a yellow plate. EI-MS (70 eV, m/z): 248 (M^+), 233 (M-CH_3), 215, 205, 191, 177. HRMS m/z (M^+): calcd. for $C_{14}H_{16}O_4$, 262.12044; found, 262.08038. UV λ_{max}^{MeOH} nm: 293, 235 (sh.), 212. IR υ_{max}^{KBr} cm^{-1}: 2927, 2854, 1733, 1635, 1610, 1465, 1433, 1290, 1238, 1203, 1141, 1110, 790, 763. ^{1}H-NMR ($CDCl_3$, 270 MHz) δ: 1.75 (3H, s, $CH_3C=CH_2$), 2.52 (3H, s, CH_3CO), 2.85 (1H, dd, J=15.0, 7.5 Hz, CH_2CH-O-), 3.21 (1H, dd, J=15.0, 9.5 Hz, -CH_2CH-O-), 3.85 (3H, s, Ar-O-CH_3) 4.85 (1H, s, $H_3CC=CH_2$), 5.01 (1H, s, $H_3CC=CH_2$), 5.23 (1H, dd, J=9.5, 7.5 Hz, -CH_2CH-O-), 5.97 (1H, s, Ar-H), 14.12 (1H, s, Ar-OH). ^{13}C-NMR ($CDCl_3$, 270 MHz) δ: 16.9, 30.8, 33.0, 55.7, 85.4, 88.1, 104.8, 106.1, 112.4, 143.4, 161.8, 164.3, 166.8, 203.2.

Isolation of euparin (**2**). Roots of *Eupatorium chinese* were collected in Kyoto Pref., extracted with hexane and concentrated under reduced pressure. The resulting extract was chromatographed on silica gel, using hexane-ethyl acetate (10:1) as the eluent, to give natural product



Scheme. Synthesis of 2,3-Dihydrobenzofuran from Methoxyphenol.
(a) Na, 1,4-dibromo-2-methyl-2-butene, ether, reflux; (b) TFA, AcOH, reflux; (c) $AlBr_3$, MeCN, H_2O.

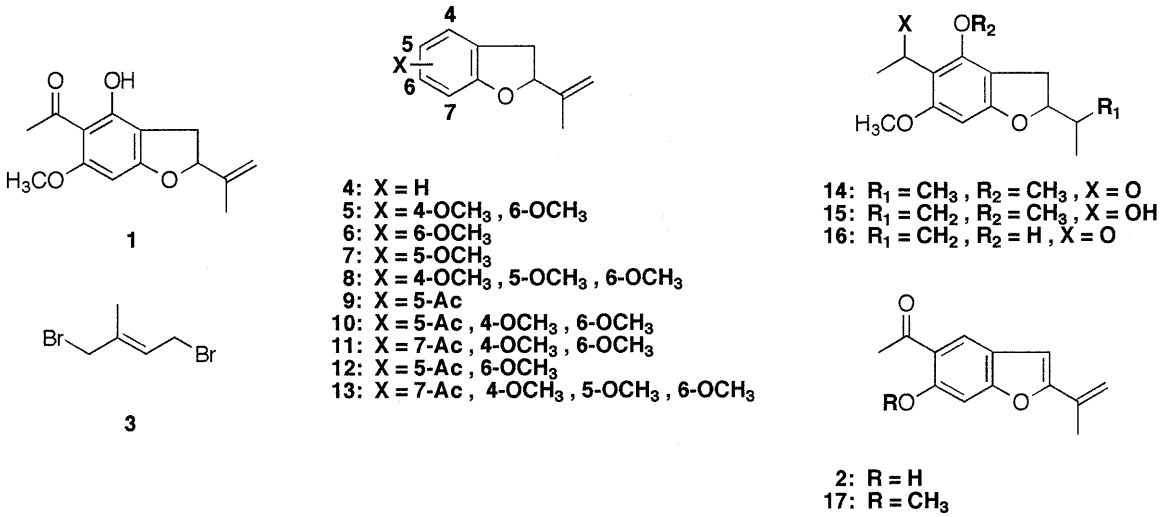


Fig. 1. Chemical Structures of the Test Compounds.

2, mp 118–119°C, as a brown plate. EI-MS (70 eV, *m/z*): 216 (M^+), 201 (M-CH_3). HRMS *m/z* (M^+): calcd. for $C_{13}H_{12}O_3$, 216.07860; found, 216.07762. ^{1}H-NMR ($CDCl_3$ 270 MHz) δ: 2.10 (3H, s, $CH_3C{=}CH_2$), 2.68 (3H, s, CH_3CO), 5.04 (1H, s, $H_3CC{=}CH_2$), 5.55 (1H, s, $H_3CC{=}CH_2$), 6.51 (1H, s, -CH=C-O-), 6.95 (1H s, Ar-H), 7.58 (1H s, Ar-H), 14.12 (1H, s, Ar-OH).

1,4-Dibromo-2-methyl-2-butene (**3**).[7] Bromine (25 g, 0.31 mol) was added dropwise to a stirred solution of isoprene (10.63 g, 0.15 mol) in CCl_4 (18.8 ml) over 8 h while keeping the temperature below 0°C. The mixture was successively washed with saturated aqueous $NaHCO_3$, distilled water and brine, and then dried over anhydrous $MgSO_4$. Filtration followed by evaporation under reduced pressure gave 1,4-dibromo-2-methyl-2-butene **3** (45.6 g, 64.5%) as a pale yellow liquid. EI-MS (70 eV, *m/z*): 228 (M^+), 149, 147 (M-Br). ^{1}H-NMR ($CDCl_3$, 270 MHz) δ: 1.88 (3H, s, CCH_3), 4.05 (2H, s, $BrCH_2$), 4.07 (2H, d, J=8.1 Hz, $BrCH_2$), 5.95 (1H, t, J=8.1 Hz, CH_2CH=).

2-Isopropenyl-2,3-dihydrobenzofuran (**4**). A solution of phenolate was prepared by mixing phenol (0.79 g, 8.4 mmol) and sodium (0.77 g, 33.6 mmol) in ether at reflux for 0.5 h. Compound **3** (2 g, 8.77 mmol) was added, and the mixture was stirred under reflux for 4.5 h. After removing the ether and additing ethanol to the residual sodium, the mixture was acidified with 10% HCl, extracted with ether, and dried over anhydrous Na_2SO_4. After filtration, evaporation was carried out under reduced pressure. The ether was removed, and the rsulting residue was chromatographed in a silica gel column, using hexane-ethyl acetate (20:1) as the eluent, to give **4** (83 mg, 6.2%) as a colorless oil. EI-MS (70 eV, *m/z*): 160 (M^+), 145 (M-CH_3). HRMS *m/z* (M^+): calcd. for $C_{11}H_{12}O$, 160.08876; found, 160.06502. ^{1}H-NMR ($CDCl_3$, 270 MHz) δ: 1.87 (3H, s, $CH_3C{=}CH_2$), 3.14 (1H, dd, J=15.5, 9.0 Hz, CH_2CH-O-), 3.43 (1H, dd, J=15.5, 9.5 Hz, -CH_2CH-O-) 5.01 (1H, s, $H_3CC{=}CH_2$), 5.20 (1H, s, $H_3CC{=}CH_2$), 3.43 (1H, dd, J=9.5, 9.0 Hz, -CH_2CH-O-), 6.91 (1H d, J=8.0 Hz, Ar-H), 6.95 (1H d, J=7.0 Hz, Ar-H), 7.22 (1H dd, J=8.0, 6.5 Hz, Ar-H), 7.24 (1H dd, J=7.0, 6.5 Hz, Ar-H).

4,6-Dimethoxy-2-isopropenyl-2,3-dihydrobenzofuran (**5**). **5** was obtained by the method just described from 3,5-dimethoxyphenol (1.68 g, 10.91 mmol) and **3** (2.49 g, 10.91 mmol) in the presence of sodium in ether under reflux for 4.5 h. Chromatography in a silica gel column, using hexane-ethyl acetate (10:1) as the eluent, gave **5** (375 mg, 15.6%) as a colorless oil. EI-MS (70 eV, *m/z*): 220 (M^+), 205 (M-CH_3). HRMS *m/z* (M^+): calcd. for $C_{13}H_{16}O_3$, 220.10988; found, 220.08970. ^{1}H-NMR ($CDCl_3$, 270 MHz) δ: 1.87 (3H, s, $CH_3C{=}CH_2$), 3.00 (1H, dd, J=15.0, 8.0 Hz, CH_2CH-O-), 3.33 (1H, dd, J=15.0, 9.5 Hz, -CH_2CH-O-), 3.87 (3H, s, Ar-O-

CH_3), 3.89 (3H, s, Ar-O-CH_3) 4.98 (1H, s, $H_3CC{=}CH_2$), 5.17 (1H, s, $H_3CC{=}CH_2$), 5.28 (1H, dd, J=9.5, 8.0 Hz, -CH_2CH-O-), 6.11 (1H, d, J=2.0 Hz, Ar-H), 6.18 (1H, d, J=2.0 Hz, Ar-H).

6-Methoxy-2-isopropenyl-2,3-dihydrobenzofuran (**6**). By the method just described, **6** was obtained from 3-methoxyphenol (1 g, 10.64 mmol) and **3** (2.43 g, 10.64 mmol) in the presence of sodium in ether under reflux for 4.5 h. Chromatography in a silica gel column, using hexane-ethyl acetate (10:1) as the eluent, gave **6** (145 mg, 7.2%) as a yellow oil. EI-MS (70 eV, m/z): 190 (M^+), 175 (M-CH_3). HRMS m/z (M^+): calcd. for $C_{12}H_{14}O_2$, 190.09932; found, 190.11160. ^{1}H-NMR ($CDCl_3$, 270 MHz) δ: 1.75 (3H, d, J=0.5 Hz, $CH_3C{=}CH_2$), 2.95 (1H, dd, J=15.0, 8.0 Hz, CH_2CH-O-), 3.25 (1H, dd, J=15.0, 9.5 Hz, -CH_2CH-O-), 3.75 (3H, s, Ar-O-CH_3), 4.88 (1H, s, $H_3CC{=}CH_2$), 5.06 (1H, d, J=0.5 Hz, $H_3CC{=}CH_2$), 5.17 (1H, dd, J=9.5, 8.0 Hz, -CH_2CH-O-), 6.38 (1H d, J=7.5 Hz, Ar-H), 6.40 (1H, s, Ar-H), 7.01 (1H, d, J=7.5 Hz, Ar-H).

5-Methoxy-2-isopropenyl-2,3-dihydrobenzofuran (**7**). By the method jsut described, **7** was obtained from 4-methoxyphenol (1.04 g, 9.63 mmol) and **3** (2 g, 8.77 mmol) in the presence of sodium in ether under reflux for 4.5 h. Chromatography in a silica gel column, using hexane-ethyl acetate (7:1) as the eluent, gave **7** (375 mg, 22.5%) as a colorless oil. EI-MS (70 eV m/z): 190 (M^+), 175 (M-CH_3). HRMS m/z (M^+): calcd. for $C_{12}H_{14}O_2$, 190.09932; found, 190.12203. ^{1}H-NMR ($CDCl_3$, 270 MHz) δ: 1.78 (3H, s, $CH_3C{=}CH_2$), 3.02 (1H, dd, J=15.5, 8.0 Hz, CH_2CH-O-), 3.31 (1H, dd, J=15.0, 9.0 Hz, -CH_2CH-O-), 3.80 (3H, s, Ar-O-CH_3), 4.90 (1H, s, $H_3CC{=}CH_2$), 5.08 (1H, s, $H_3CC{=}CH_2$), 5.14 (1H, dd, J=9.0, 8.0 Hz, -CH_2CH-O-), 6.65 (1H, dd, J=8.5, 2.0 Hz, Ar-H), 6.69 (1H, d, J=8.5 Hz, Ar-H), 6.74 (1H, d, J=2.0 Hz, Ar-H).

4,5,6-Trimethoxy-2-isopropenyl-2,3-dihydrobenzofuran (**8**). By the method just described, **8** was obtained from 3,4,5-trimethoxyphenol (1.62 g, 8.77 mmol) and 3 (2 g, 8.77 mmol) in the presence of sodium in ether at reflux for 16 h. Chromatography in a silica gel column, using hexane-ethyl acetate (2:1) as the eluent, gave **8** (91 mg, 4.2%) as a pale yellow oil. EI-MS (70 eV, m/z): 250 (M^+), 235 (M-CH_3). HRMS m/z (M^+): calcd. for $C_{14}H_{18}O_4$, 250.12044; found, 250.13261. ^{1}H-NMR ($CDCl_3$, 270 MHz) δ: 1.75 (3H, s, $CH_3C{=}CH_2$), 3.00 (1H, dd, J=15.5, 8.0 Hz, CH_2CH-O-), 3.33 (1H, dd, J=15.0, 9.5 Hz, -CH_2CH-O-), 3.75 (3H, s, Ar-O-CH_3), 3.78 (3H, s, Ar-O-CH_3), 3.90 (3H, s, Ar-O-CH_3), 4.88 (1H, s, $H_3CC{=}CH_2$), 5.05 (1H, s, $H_3CC{=}CH_2$), 5.12 (1H, dd, J=9.5, 8.0 Hz, -CH_2CH-O-), 6.18 (1H, s, Ar-H).

5-Acetyl-2-isopropenyl-2,3-dihydrobenzofuran (*racemic tremetone*; **9**). Trifluoroacetic anhydride (0.33 ml, 2.4 mmol) was added to a solution of **4** (128 mg, 0.8 mmol) in acetic acid (0.1 ml, 1.6 mmol) in an ice-water bath. The mixture was allowed to stand for 1.5 h at room temperature. After the reaction, the mixture was treated with ice-water, made alkaline with an aqueous solution of sodium hydrogencarbonate, and extracted with ether. The ethereal fraction was dried over anhydrous Na_2SO_4. After removing the ether, the resulting oil was subjected to chromatography in a silica gel column, using hexane-ethyl acetate (2:1) as the eluent, to give **9** (55 mg, 33.8%), mp 39–40°C, as white crystals. EI-MS (70 eV, m/z): 202 (M^+), 187 (M-CH_3). HRMS m/z (M^+): calcd. for $C_{13}H_{14}O_2$, 202.09932; found, 202.09247. ^{1}H-NMR ($CDCl_3$, 270 MHz) δ: 1.74 (3H, s, $CH_3C{=}CH_2$), 2.52 (3H, s, CH_3CO), 3.04 (1H, dd, J=16.0, 8.5 Hz, CH_2CH-O-), 3.37 (1H, dd, J=15.0, 9.5 Hz, -CH_2CH-O-), 4.92 (1H, s, $H_3CC{=}CH_2$), 5.07 (1H, s, $H_3CC{=}CH_2$), 5.25 (1H, dd, J=9.5, 8.5 Hz, -CH_2CH-O-), 6.81 (1H, d, J=7.5 Hz, Ar-H), 7.79 (1H, d, J=7.5 Hz, Ar-H), 7.80 (1H, s, Ar-H).

5-Acetyl-2-isopropenyl-4,6-dimethoxy-2,3-dihydrobenzofuran (*methylremirol*; **10**) *7-Acetyl-2-isopropenyl-4,6-dimethoxy-2,3-dihydrobenzofuran* (**11**). Trifluoroacetic anhydride (0.53 ml, 3.81 mmol) was added to a solution of **5** (269 mg, 1.22 mmol) in acetic acid (0.14 ml, 2.5 mmol) in an ice-water bath. The mixture was allowed to stand for 3 h at room temperature. After the reaction, the mixture was treated with ice-water, made alkaline with aqueous $NaHCO_3$, and extracted with ether. The ethereal fraction was dried over anhydrous Na_2SO_4. After removing the ether, the resulting yellow oil was subjected to chromatography in a silica gel column, using hexane-ethyl acetate (2:1) as the eluent, to give **10** (76 mg, 23.8%) and **11** (75 mg, 23.5%) each as a colorless oil. **10**. EI-MS (70 eV, m/z): 262 (M^+), 247 (M-CH_3). HRMS m/z (M^+): calcd. for $C_{15}H_{18}O_4$, 262.12044; found, 262.08038. ^{1}H-NMR ($CDCl_3$, 270 MHz) δ: 1.78 (3H, s, $CH_3C{=}CH_2$), 2.46 (3H, s, CH_3CO), 3.07 (1H, dd, J=15.0, 8.0 Hz, CH_2CH-O-), 3.42 (1H, dd, J=15.0, 9.5 Hz, -CH_2CH-O-), 3.77 (3H, s, Ar-O-CH_3), 3.84 (3H, s, Ar-O-CH_3), 4.93 (1H, s, $H_3CC{=}CH_2$), 5.08 (1H, s, $H_3CC{=}CH_2$), 5.20 (1H, dd, J=9.5, 8.5 Hz, -CH_2CH-O-), 6.18 (1H, s, Ar-H). **11**. EI-MS (70 eV, m/z): 262 (M^+), 247 (M-CH_3). HRMS m/z (M^+): calcd. for $C_{15}H_{18}O_4$, 262.12044; found, 262.13271. ^{1}H-NMR ($CDCl_3$, 270 MHz) δ: 1.77 (3H, s, $CH_3C{=}CH_2$), 2.51 (3H, s, CH_3CO), 2.84 (1H, dd, J=15.0, 8.0 Hz, CH_2CH-O-), 3.20 (1H, dd, J=15.0, 9.5 Hz, -CH_2CH-O-), 3.74 (3H, s, Ar-O-CH_3), 3.83 (3H, s, Ar-O-CH_3), 4.87 (1H, s, $H_3CC{=}CH_2$), 5.03 (1H, s, $H_3CC{=}CH_2$), 5.25 (1H, dd, J=9.5, 8.5 Hz, -CH_2CH-O-), 5.96 (1H, s, Ar-H).

5-Acetyl-2-isopropenyl-6-methoxy-2,3-dihydrobenzofuran (**12**).[8] Trifluoroacetic anhydride (0.09 ml, 0.68 mmol) was added to a solution of **6** (43 mg, 0.23 mmol) in acetic acid (0.04 ml, 0.45 mmol) in an ice-water bath. The mixture was allowed to stand for 1.5 h at room temperature. After the reaction, the mixture was treated with ice-water, made alkaline with saturated $NaHCO_3$, and extracted with ether. The ethereal fraction was dried over anhydrous Na_2SO_4. After removing the ether, the resulting solid was recrystallized from hexane to give **12**

(13 mg, 24.8%), mp 101–102°C, as yellow crystals. EI-MS (70 eV, *m/z*): 232 (M^+), 217 (M-CH_3). HRMS *m/z* (M^+): calcd. for $C_{14}H_{16}O_3$, 232.10988; found, 232.11492. ^{1}H-NMR ($CDCl_3$, 270 MHz) δ: 1.84 (3H, s, $CH_3C=CH_2$), 2.66 (3H, s, CH_3CO), 3.06 (1H, dd, J=15.0, 8.0 Hz, CH_2CH-O-), 3.39 (1H, dd, J=15.0, 9.5 Hz, -CH_2CH-O-), 3.97 (3H, s, Ar-O-CH_3), 5.02 (1H, s, $H_3CC=CH_2$), 5.17 (1H, s, $H_3CC=CH_2$), 5.35 (1H, dd, J=9.5, 8.5 Hz, -CH_2CH-O-), 6.52 (1H, s, Ar-*H*), 7.78 (1H, s, Ar-*H*).

7-Acetyl-2-isopropenyl-4,5,6-trimethoxy-2,3-dihydrobenzofuran (**13**). Trifluoroacetic anhydride (0.11 ml, 0.81 mmol) was added to a solution of **8** (67 mg, 0.27 mmol) in acetic acid (0.03 ml, 0.54 mmol) in an ice-water bath. The mixture was then allowed to stand for 3 h at room temperature. After the reaction, the mixture was treated with ice-water, made alkaline with saturated $NaHCO_3$, and extracted with ether. The ethereal fraction was dried over anhydrous Na_2SO_4. After removing the ether, the resulting yellow oil was subjected to chromatography in a silica gel column, using hexane-ethyl acetate (3:1) as the eluent, to give **13** (26 mg, 33.2%) as a yellow oil. EI-MS (70 eV *m/z*): 292 (M^+), 277 (M-CH_3). HRMS *m/z* (M^+): calcd. for $C_{16}H_{20}O_5$, 292.13100; found, 292.13410. ^{1}H-NMR ($CDCl_3$, 270 MHz) δ: 1.76 (3H, s, $CH_3C=CH_2$), 2.54 (3H, s, CH_3CO), 3.03 (1H, dd, J=15.0, 8.0 Hz, CH_2CH-O-), 3.37 (1H, dd, J=15.0, 9.5 Hz, -CH_2CH-O-), 3.78 (3H, s, Ar-O-CH_3), 3.86 (3H, s, Ar-O-CH_3), 3.94 (3H, s, Ar-O-CH_3), 4.90 (1H, s, $H_3CC=CH_2$), 5.06 (1H, s, $H_3CC=CH_2$), 5.20 (1H, dd, J=9.5, 8.5 Hz, -CH_2CH-O-).

5-Acetyl-2-isopropyl-4,6-dimethoxy-2,3-dihydrobenzofuran (**14**). **10** (30 mg, 0.125 mmol) was hydrogenated in ethanol over 10% palladium on charcoal (30 mg) at room temperature. After the uptake of 1 mole of H_2, the solution was passed through a glass filter, and the resulting oil was subjected to preparative TLC, using hexane-ethyl acetate (3:1) as the eluent, to give **14** (20 mg, 66.1%), mp 84–85°C, as white crystals. EI-MS (70 eV, *m/z*): 264 (M^+), 249 (M-CH_3). HRMS *m/z* (M^+): calcd. for $C_{15}H_{20}O_4$, 264.13608; found, 264.14309. ^{1}H-NMR ($CDCl_3$, 270 MHz) δ: 0.94 (3H, d, J=7.0 Hz, CH_3CH-CH_3), 1.00 (3H, d, J=7.0 Hz, CH_3CH-CH_3), 1.92 (1H, m, CH_3CH-CH_3), 2.43 (3H, s, CH_3CO), 2.93 (1H, dd, J=15.0, 8.5 Hz, CH_2CH-O-), 3.22 (1H, dd, J=15.0, 9.5 Hz, -CH_2CH-O-), 3.72 (3H, s, Ar-O-CH_3), 3.81 (3H, s, Ar-O-CH_3), 4.51 (1H, ddd, J=9.5, 8.5, 7.0 Hz, -CH_2CH-O-), 6.12 (1H, s, Ar-*H*).

5-(1-Hydroxyethyl)-2-isopropenyl-4,6-dimethoxy-2,3-dihydrobenzofuran (**15**). Lithium aluminum hydride (1.71 ml, 0.045 mmol) was added to a solution of **10** (10 mg, 0.045 mmol) in ether. The mixture was allowed to stand for 1 h at room temperature. After the reaction, the mixture was treated with water (1 ml) and dried over anhydrous Na_2SO_4. After removing the ether, the resulting oil was subjected to preparative TLC, using hexane-ethyl acetate (3:1) as the eluent, to give **15** as one diastereomer (4 mg, 33.7%), as a colorless oil. EI-MS (70 eV, *m/z*): 264 (M^+), 249 (M-CH_3). HRMS *m/z* (M^+): calcd. for $C_{15}H_{20}O_4$, 264.13608; found, 264.13451. ^{1}H-NMR ($CDCl_3$, 270 MHz) δ: 1.59 (3H, d, J=7.0 Hz, CH_3CHOH), 1.87 (3H, s, $CH_3C=CH_2$), 3.18 (1H, dd, J=15.0, 8.5 Hz, CH_2CH-O-), 3.55 (1H, dd, J=15.0, 9.0 Hz, -CH_2CH-O-), 3.77 (1H, s, CHO*H*), 3.90 (3H, s, Ar-O-CH_3), 3.97 (3H, s, Ar-O-CH_3), 5.02 (1H, d, J=1.0 Hz, $H_3CC=CH_2$), 5.18 (1H, s, $H_3CC=CH_2$), 5.27 (1H, dd, J=9.0, 8.5 Hz, -CH_2CH-O-), 5.28 (1H, q, J=7.0 Hz, CH_3CHOH), 6.32 (1H, s, Ar-*H*). The other diastereomer: ^{1}H-NMR ($CDCl_3$, 270 MHz) δ: 1.59 (3H, d, J=7.0 Hz, CH_3CHOH), 1.86 (3H, s, $CH_3C=CH_2$), 3.16 (1H, dd, J=15.0, 8.5 Hz, CH_2CH-O-), 3.53 (1H, dd, J=15.0, 9.0 Hz, -CH_2CH-O-), 3.77 (1H, s, CHO*H*), 3.90 (3H, s, Ar-O-CH_3), 3.95 (3H, s, Ar-O-CH_3), 5.01 (1H, d, J=1.0 Hz, $H_3CC=CH_2$), 5.18 (1H, s, $H_3CC=CH_2$), 5.25 (1H, dd, J=9.0, 8.5 Hz, -CH_2CH-O-), 5.28 (1H, q, J=7.0 Hz, CH_3CHOH), 6.32 (1H, s, Ar-*H*).

5-Acetyl-2-isopropenyl-4-hydroxy-6-methoxy-2,3-dihydrobenzofuran (*racemic remirol*; **16**).[9] **10** (14 mg, 0.053 mmol) was dissolved in a 10% (w/v) solution of anhydrous aluminum bromide in acetonitrile (0.87 ml) while stirring, and the solution was warmed at 70°C for 10 min to give **16** (4 mg, 30.2%) as a colorless oil. EI-MS (70 eV, *m/z*): 248 (M^+), 233 (M-CH_3). HRMS *m/z* (M^+): calcd. for $C_{14}H_{16}O_4$, 248.10480; found, 248.13799. ^{1}H-NMR ($CDCl_3$, 270 MHz) δ: 1.75 (3H, s, $CH_3C=CH_2$), 2.59 (3H, s, CH_3CO), 2.90 (1H, dd, J=15.0, 7.5 Hz, CH_2CH-O-), 3.25 (1H, dd, J=15.0, 9.5 Hz, -CH_2CH-O-), 3.86 (3H, s, Ar-O-CH_3) 4.90 (1H, s, $H_3CC=CH_2$), 5.02 (1H, s, $H_3CC=CH_2$), 5.26 (1H, dd, J=9.5, 7.5 Hz, -CH_2CH-O-), 5.97 (1H, s, Ar-*H*), 14.09 (1H, s, Ar-O*H*).

5-Acetyl-2-isopropenyl-6-methoxy-benzofuran (*methyl euparin*; **17**).[6] Potassium carbonate (26 mg, 0.185 mmol) and dimethyl sulfate (23 mg, 0.185 mmol) were added to a solution of **2** (40 mg, 0.185 mmol) in acetone. The mixture was allowed to stand for 3 days under reflux. After the reaction, the mixture was washed with water and extracted with ether. The extract was dried over anhydrous Na_2SO_4 under reduced pressure. The resulting solid was recrystallized from hexane to give **17** (34 mg, 80.0%), mp 48–49°C, as a yellow plate. EI-MS (70 eV *m/z*): 230 (M^+), 215 (M-CH_3). HRMS *m/z* (M^+): calcd. for $C_{14}H_{14}O_3$, 230.09424; found, 230.07514. ^{1}H-NMR ($CDCl_3$, 270 MHz) δ: 2.10 (3H, s, $CH_3C=CH_2$), 2.64 (3H, s, CH_3CO), 3.94 (3H, s, Ar-O-CH_3) 5.15 (1H, s, $H_3CC=CH_2$), 5.72 (1H, s, $H_3CC=CH_2$), 6.57 (1H, s, -CH=CH-O-), 7.02 (1H s, Ar-*H*), 7.93 (1H s, -CH=CH-O-).

Evaluation of insect antifeedant activity. The dual-choice leaf disk assay was employed. Disks of 2 cm in diameter were prepared with a cork borer from fresh leaves of sweet potato (*Ipomoea batatas*) that had been cultivated at Kinki University farm (Nara Pref.) without

agrochemicals.

Two disks were treated with each test compound in an acetone solution and two with acetone alone as controls. The 4 disks were then placed in the form of a cross in the same Petri dish. After completely removing the solvent, 15 larvae (3rd instar) were released into the dish. All dishes were then kept in an insect rearing room at 26.5°C for 2–5 h. The partially consumed leaf disks were taped on to copy paper for monotone data conversion. The monotone data were photocopied and confirmed to contain no errors, and then converted to digital data with a digital scanner. A digital data analysis was performed with a Macintosh computer, using the public-domain NIH Image program. For each experiment, the data for an intact disk were measured and compared to these for a damaged disk. To measure the activity of each compound, we adopted the following antifeedant index: AFI = % of treated disks consumed / (% of treated disks consumed + % of control disks consumed) × 100. When the same amount was consumed of both a treated and control disk, the AFI value was set at 50.[10] The ED_{50} values of the test compounds were calculated with a computer by the probit method.

Results and Discussion

Table 1 shows the ED_{50} values for those compounds showing a significant correlation between the dose and feeding response. The diversity of antifeedant activity of the tested benzofuran derivatives indicated some form of structure-activity relationship. With an ED_{50} value of 5.4×10^{-9} mol/cm^2, 7-acetyl-2-isopropenyl-4,6-dimethoxy-2,3-dihydrobenzofuran (**11**) was among the most active of insect antifeedants yet recorded.

We prepared benzofuran derivatives with various structural differences to evaluate the effects on the feeding response of *S. litura* larvae (Fig. 1). In all assays, the test compounds were evaluated as racemic versions, and the results (Tables 1 and 2) indicate that certain substituents affected the antifeedant activity (excluding the natural compounds and their derivatives).

Effect of oxy-substituents on the benzene ring

The chemical features of **4** show that all of the substituents are hydrogen on its aromatic ring, except for the furan ring; this compound has a weak inhibitory effect on insect feeding. However, when a methoxy group was introduced into the aromatic ring, insect feeding was markedly inhibited. Further, the number of methoxy groups was related to this activity. A comparison of the methoxy derivatives (**5–8**) revealed the most effective number of methoxy groups to be two or three. The evaluation of **9** revealed that substitution with an acetyl group also increased the antifeedant activity. This compound (**9**), tremetone, has exhibited toxicity toward fish and is a constituent of the *Eupatorium* genus.[11] Interestingly, in spite of the same substituents being present on the aromatic ring, two acetyl derivatives (**10** and **11**) had completely different antifeedant activity and polarity as detected by the TLC analysis. Their physicochemical properties seem to have significantly influenced their inhibitory effects on insect feeding. The importance of introducting an acetyl group at the 7-position of the 2,3-dihydrobenzofuran ring is also supported by the results for **13**. On the other hand, the introduction of a hydrophilic functional group, *e.g.* a hydroxyethyl derivative (**15**), tended to decrease the antifeedant activity. Similar observations have been reported for the structure-activity relationship of acetylchromenes in the desert sunflower, *Encelia* sp., the hydroxylated metabolites showing less insecticidal activity.[12] In addition, substitution of an acetyl or methoxy group on the aromatic ring in capillin derivatives resulted in feeding inhibition of *Pieris rapae*.[13]

Effects of the hydrogenated derivatives and others

Changes to the chemical structure of the coumarans (2,3-dihydrobenzofuran) were evaluated. A comparison between **10** and **14** revealed that reducing the 2-isopropenyl group caused a slight decrease in antifeedant activity. Similarly, inhibited mitochondrial respiration was apparent by reducing the isopropenyl group of rotenone, including the coumaran (2,3-di-

Table 1. Insect Antifeedant Activity of 2,3-Dihydrobenzofuran Derivatives against *Spodoptera litura*

Compound	Substituent (on the benzene ring)	R_f value[b]	Antifeedant activity[a] (mol/cm^2)	
			ED_{50}	pED_{50}
4	none	0.92	1.1×10^{-6}	5.96
5	4,6-dimethoxy	0.82	5.3×10^{-8}	7.28
6	6-methoxy	0.86	6.3×10^{-7}	6.20
7	5-methoxy	0.84	1.3×10^{-7}	6.89
8	4,5,6-trimethoxy	0.68	5.6×10^{-8}	7.25
9	5-acetyl	0.61	7.4×10^{-8}	7.13
10	5-acetyl-4,6-dimethoxy	0.52	2.8×10^{-8}	7.55
11	7-acetyl-4,6-dimethoxy	0.24	5.4×10^{-9}	8.27
12	5-acetyl-6-methoxy	0.62	4.2×10^{-8}	7.38
13	7-acetyl-4,5,6-trimethoxy	0.52	1.6×10^{-8}	7.80

[a] pED_{50}, the logarithm of the reciprocal of ED_{50}, which is the molar dose on the treated leaf disk consumed by 50%, was used as an index of insect antifeedant activity.

[b] The R_f value of each test compound was determined by using a hexane-ethyl acetate solvent system (5:2) on a silica-gel TLC plate.

Table 2. Insect Antifeedant Activity of Acetylbenzofuran Derivatives against *Spodoptera litura*

Compound	Structural Feature	R_f value[b]	Antifeedant activity[a] (mol/cm^2)	
			ED_{50}	pED_{50}
1	natural remirol (natural compound)	0.51	1.3×10^{-7}	6.89
2	euparin (natural compound)	0.76	1.2×10^{-6}	5.92
14	reduction of isopropenyl group	0.58	7.6×10^{-8}	7.12
15	reduction of acetyl group	0.83	1.1×10^{-7}	6.96
16	racemic remirol	0.51	7.1×10^{-8}	7.15
17	methyl euparin	0.66	6.1×10^{-8}	7.21

[a] pED_{50}, the logarithm of the reciprocal of ED_{50}, which is the molar dose on the treated leaf disk consumed by 50%, was used as an index of insect antifeedant activity.
[b] The R_f value of each test compound was determined by using a hexane-ethyl acetate solvent system (5:2) on a silica-gel TLC plate.

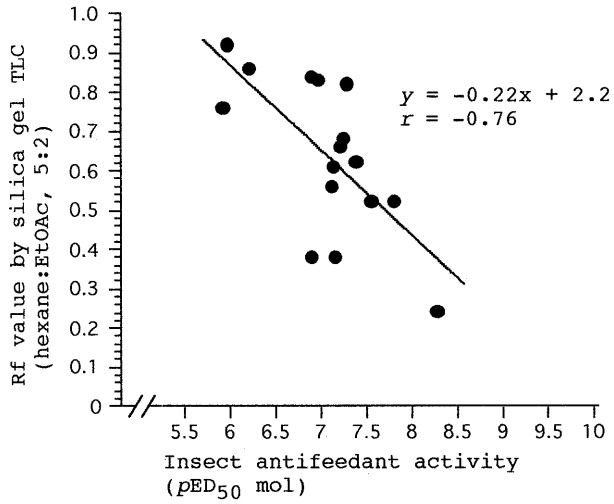


Fig. 2. Correlation between the Antifeedant Activity and R_f Value by TLC.
The relative antifeedant activity is shown as pED_{50} in mol units. The TLC analysis was conducted with a hexane-ethyl acetate solvent system.

hydrobenzofuran) moiety in the chemical structure.[14] The 2,3-dihydrofuran derivative (**12**) did not show any significantly different activity from the furan derivatives (**17**). Thus, the 2,3-dihydrobenzofuran moiety may not affect the insect antifeedant activity. The comparison between the natural compound, remirol (**1**), and racemic remirol (**16**) indicated the effect of chirality of the 2-position on the 2,3-dihydrofuran ring on insect feeding (Table 2). These antifeedant activities reveal **16** to be slightly more active than natural compound **1**. This difference in antifeedant activity may have been caused by different physical properties; natural remirol (**1**) is crystalline, but the racemic version is oily at room temperature. Similarly, Lane *et al.* have reported that isoflavones show antifeedant activity against the leaf beetle that was dependent on chiral orientation.[15]

Relationship between the lipophilicity and inhibitory effect on insect feeding

There appeared to be a relationship between the lipophilicity and antifeedant activity of the test compounds (Fig. 2). In this study, we adopted the R_f value obtained from the TLC analysis with the same solvent because the compounds had a similar chemical structures and polarity. The most effective compounds in the antifeedant bioassay showed high polarity (a small R_f value) by the TLC analysis. This suggested that the compounds required a certain degree of lipophilicity in order to be transported to the receptor site or target organ. In general, even if an agent has potential activity, it will be ineffective when it is unable to penetrate biological membranes.

Based on the variation in inhibitory activity of 2,3-dihydrobenzofuran toward insect feeding, we conclude that the introduction of an acetyl or methoxy group on the aromatic ring produced effective antifeedant activity; on the contrary, conversion to a hydroxyl group decreased the biological activity. Moreover, the position of the substituted acetyl group appreciably affected the activity. There is a tendency for the R_f values of the test compounds to be related to their activity. Further studies are necessary to clarify these points. Many natural compounds are known to act as insect antifeedants.[16] The majority of these natural compounds have an ether moiety in a furan or pyran ring. Our test compounds all had a furan ring and little phytotoxicity. This feature of low phytotoxicity should be helpful in the development of agrochemicals for crops. However, some of these compounds also had an acute toxic effect on the water flea. The toxicity of **11** revealed an LD_{50} value of 9.1 ppm at 12 h after treatment against water flea (*Daphnia magna*). However, these details regarding the relationship between fish toxicity and antifeedant activity are now under more detailed investigation.

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