Electrochemical Synthesis of Dihydrobenzofurans and Evaluation of Their Insect Antifeedant Activities

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Abstract: Electrochemically synthesized dihydrobenzofurans were evaluated for their insect antifeedant activities against phytophagous insects. They were prepared through the coupling reactions of various alkenes with a phenoxy cation generated by oxidation near the cathode in the electrolytic reaction. The insect antifeedant activities of these synthetic dihydrobenzofurans were evaluated in the common cutworm (Spodoptera litura) and diamond back moth (Plutella xylostella) with the dual choice leaf disk bioassay method. The insect antifeedant activities of most of the acetophenone-type dihydrobenzofurans were strong, while those of derivatives with a t-buty1 group were weaker. The biological activities in insect species differed with the structural features of the compounds.

Key words: dihydrobenzofuran, electrochemical synthesis, insect antifeedant

1 INTRODUCTION
Various dihydrobenzofurans and lignans with a saturated furan ring are found in numerous plants and exhibit various biological activities1. On the other hand, dihydrobenzofuran-containing insecticides, such as carbofuran and benfuracarb, are commercially available. To search for lead compounds of agricultural chemicals, we focused on wild plants that should have more an effective chemical defense than crops protected by agricultural chemicals. Although many reports have been published on the chemoprotection of plants from wild plants, we investigated a wild plant, Cyperus spp. We previously reported that these plant species produced the insect antifeedant quinones, cyperquinones, and its precursor dihydrobenzofuran, remirol also exhibited similar biological activity2. The antifeeding action of dihydrobenzofuran on the larvae of Spodoptera litura will be briefly described. We revealed that the introduction of an acetyl group to the benzene ring of dihydrobenzofuran was the most effective strategy for increasing insect antifeedant activity3. On the other hand, previous studies described the synthesis of dihydrobenzofurans by sigmatropic rearrangement4, acyl migration5, and electrochemically6. The aims of the present study were to prepare insect antifeedant dihydrobenzofurans by electrochemical synthesis, and evaluate structure-activity relationship (SAR) using dihydrobenzofurans synthesized for this purpose.

2 EXPERIMENTAL

2.1 Materials
NMR spectra were measured on JEOL model EX-270 containing TMS as an internal standard. MS spectra were measured on Shimadzu QP-1000 and GCMS 9100-MK. In the reaction vessel, the potential was constantly controlled with a potentiostat (HA-10 Hokuto Denko Corp., Tokyo) using two glass carbon electrodes at room temperature under a nitrogen atmosphere. The reaction mixture was purified by silica gel column chromatography and recrystallization.

2.2 Chemicals
Hexane and ethyl acetate were purchased from Wako Pure Chemical Industries and used without purification. A silica gel for column chromatography was purchased from Fuji Silysia Chemical Ltd.. TLC plates were purchased from MERCK Co..

2.3 Typical procedure for the electrochemical synthesis of dihydrobenzofurans
The general procedure for electrochemical reactions involved the addition of p-methoxyphenol (1.0 g, 8.0 mmol), 2-methyl-2-butene (1.12 g, 16.0 mmol), and sodium acetate (65 mg, 0.8 mmol) to LiClO4 (16g, 150 mmol) in CH3NO2 (50 ml) solution. The reaction cell equipped with the two bare glassy carbon felts and silver-silver chloride electrode as SCE was capped with a cork. Under a nitrogen atmosphere,
the electrolysis reaction was carried out for 48 hours under the control of a potentiostat through an oxidation reaction at an appropriate oxidation potential of 600 mV. After the reaction was completed, the reaction mixture was separated between AcOEt and water. The organic layer was dried over anhydrous MgSO4. After filtration and evaporation under reduced pressure, the residue was purified by silica gel column chromatography using a hexane-AcOEt solvent system, followed by recrystallization to obtain the cycloducts, dihydrobenzofuranos.

2.4 Evaluation of insect antifeedant activities of prepared dihydrobenzofurans

Eighteen benzofurans (1-18) were prepared by electrochemical oxidation for the SAR of insect antifeedant activities against common cutworms. The yields of these synthesizes were between ca. 7-90%, and were dependent on the combination pattern between phenols and alkenes. The dihydrobenzofurans tested were designed based on previous SAR findings, in which the introduction of an acetyl group at the 7-position of the benzene ring and the presence of a methoxy group in the dihydrobenzofuran moiety were shown to be advantageous for insect antifeedant activity in common cutworms. Therefore, the prepared dihydrobenzofuran series had a hydrogen, acetyl, and t-butyl group at the 7-position. The methylenedioxy structure is found in many physiologically active substances, such as piperine derivatives (insecticidal compounds) and piperonyl butoxide (insect growth inhibitor).

5-Methoxy-2,2,3-trimethyl-2,3-dihydrobenzofuran (1), HRMS m/z 192.11503 (M+ C17H18O2 requires 192.11496); EIMS 268 (M+, 100); 1H NMR (CDCl3, 270 MHz) δ (ppm): 7.36 (5H, m), 7.23 (1H, d, J = 3.0 Hz), 6.96 (1H, d, J = 3.0 Hz), 5.84 (1H, dd, J = 9.5, 8.0 Hz), 3.76 (3H, s), 3.61 (1H, dd, J = 16.0, 9.5 Hz), 3.17 (1H, dd, J = 16.0, 8.0 Hz), 2.65 (3H, s).

7-t-Butyl-5-methoxy-2-phenyl-2,3-dihydrobenzofuran (6), HRMS 282.09872 (M+ C17H19O2 requires 282.16198); EIMS 282 (M+, 100), 267 (99.6); 1H NMR (CDCl3, 270 MHz) δ (ppm): 7.37 (5H, m), 6.70 (1H, d, J = 2.3 Hz), 6.62 (1H, d, J = 2.3 Hz), 5.74 (1H, dd, J = 9.5, 8.5 Hz), 3.76 (3H, s), 3.57 (1H, dd, J = 15.5, 9.5 Hz), 3.11 (1H, dd, J = 15.5, 8.5 Hz), 1.39 (9H, s).

3-Methyl-5-methoxy-2-(3'4'-dimethoxyphenyl)-2,3-dihydrobenzofuran (7), HRMS 300.18012 (M+ C17H19O2 requires 300.18204); EIMS (M+ 100), 285 (65.7); 1H NMR (CDCl3, 270 MHz) δ (ppm): 7.23 (1H, d, J = 3.0 Hz), 6.96 (1H, d, J = 8.0 Hz), 6.95 (1H, s), 6.90 (1H, d, J = 3.0 Hz), 6.88 (1H, d, J = 8.0 Hz) 3.89 (3H, s), 3.88 (3H, s), 3.80 (3H, s), 3.40 (1H, dq, J = 9.0, 7.0 Hz), 2.63 (3H, s), 1.41 (3H, d, 7.0 Hz).

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2.5 Evaluation of insect antifeedant activities of dihydrobenzofurans

In the insect antifeedant activity test against common cutworms and diamond back moth larvae, leaf disks, 2 cm in diameter, were prepared with a cork borer from fresh sweet potato (Ipomoea batata cv. narutokintonoki) leaves for common cutworms or leaf disks, 1 cm in diameter, were prepared with a cork borer from fresh cabbage (Brassica oleracea) leaves for diamond back moth larvae. These vegetables for leaf disk preparation had been cultivated in the farm at Kindai University (Nara Pref., Japan). Two disks were treated with test compounds in acetone solution and two other disks were treated with aceton only as a control. These 4 disks were set in alternating positions in the same petri dish, 9.5 cm in diameter, with moistened filter paper on the bottom. After acetone had completely evaporated, 15 larvae (3rd instar) were released onto the dish. Dishes were then kept in an insect-rearing room at 26.5°C in the dark for 5–6 h. Partially consumed leaf disks were digitized using a PC scanner and converted to respective monotone data. Data were analyzed on a PC using NIH Image software (https://imagej.nih.gov/niih-image/). In each experiment, data for an intact disk were measured and compared to those of a treated disk. In order to measure the activities of the test compounds, we used an antifeedant index: AFI = % of treated disks consumed / (% of treated disks consumed + % of control disks consumed)x 100. The AFI value was converted to an inhibitory rate (%): inhibitory rate (%) = (50-AFI) × 2. The insect antifeedant activities of the tested compounds were evaluated in terms of the ED50 value for the rate of feeding inhibition calculated from the area of the leaf disk consumed. A straight line was fit to the points obtained by the bioassay and ED50 and ED95 were calculated as the dose that corresponded to the midpoint between complete inhibition and no effect.

3 RESULTS AND DISCUSSION

All dihydrobenzofurans (1-18) were synthesized by the cycloaddition of an electrically generated phenoxymonon cation to their inactivated alkene (Figs. 1 and 2). Coupling reactions were performed by initially mixing all starting materials with the relevant electrolytes in solvent using two bare glassy carbon foils as the two poles. An appropriate charge was passed using a reference electrode in the reaction vessel. The solvent and electrolyte used were nitromethane and lithium perchlorate, respectively, as described by Chiba et al.6.

The results of the insect antifeedant activity test revealed that acetophenone-type dihydrobenzofurans, having an acetyl group at the 7-position on the benzene ring, namely, 2, 5, 8, 11, and 17, but not 14, exhibited stronger insect antifeedant activities in both test insect species than
their respective congeners. The most effective compounds against common cutworms were the \( o \)-dimethoxyphenyl-attached acetophenone-type dihydrobenzofuran (8) and methylenedioxy-attached acetophenone-type dihydrobenzofuran (17), whereas that for the diamond back moth was the phenyl group-eliminated acetophenone-type dihydrobenzofuran (2). Correspondingly, the neolignan, kusunokinin, possessing a methylenedioxy and \( o \)-dimethoxyphenyl from *Aristolochia malmeana*, has been reported to exhibit insecticidal activity against the velvet bean caterpillar moth (*Anticarsia gemmatalis*) larvae. This activity by the active dihydrobenzofuran (8) was weaker against diamond back moth larvae and was completely absent for the dihydrobenzofuran (17) (Table 1). Additionally, none of the 3'-acetoxy derivatives exhibited insect antifeedant activity. In a comparison between non-stilbene (1-3) and stilbene, C6-C2-C6, types (4-18), stilbene-type dihydrobenzofurans exhibited slightly stronger activity. In a comparison of insect antifeedant activities between phenylpropanoids and lignans, lignans were more advantageous against the common cutworm than phenylpropanoids (11). The neolignans from *Piper decurrence* also exhibited insect antifeedant activities against European corn borer (*Ostrinia nubilalis*) larvae. In the present study, trimethoxy derivatives 7-9 showed the highest antifeeding action. As previously reported, epimagnolin A derived from magnolia, a polymethoxy lignan with a similar structure, shows a high growth inhibitory activity on *Drosophila* larvae. The \( o \)-dimethoxyphenyl derivative (8) exhibited stronger insect antifeedant activity than the corresponding \( o \)-methoxyhydroxy derivative (11) against common cutworms (Table 1). Wukirsari, T. *et al.* also reported that the strongest insecticidal lignan was the \( o \)-dimethoxyphenyl derivative in this SAR study using the systemic synthetic lignan series against *Musca domestica* (14). The neolignan, ococymosin from *Ocotea cymosa*, possessing a similar core to our compounds, exhibited insecticidal activities against *Aedes aegypti* and *Plasmodium falciparum* (15).

Many phytochemicals have been shown to exhibit insect antifeedant activities including alkaloids, terpenes, and phenolics (16, 17). A very well-known insect antifeedant is the limonoid, azadirachtins from the fruits of neem *Azadirachta indica*, and these natural products consist of several saturated furan rings (18). The mode of action involves the insect antifeedant inhibition of metamorphosis and insecticidal activity (19). Furthermore, aurones are a type of flavonoid that possess a benzofuranone moiety and exhibit insect antifeedant activity against the common cutworm (20), demonstrating that the benzofuran moiety enhances insect antifeedant activity. We previously reported that these plant species produced the insect antifeedant quinones, cyperaquinones, and its precursor dihydrobenzo-
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Remirol and also lead of this study, remirol exhibited insect antifeedant activity (Fig. 3). Actually, dihydrobenzofuran containing insecticides, such as carbofuran and benfura-carb, are already commercially available (Fig. 4). Like aflatoxin B (mycotoxin), benzofurans become cytotoxic and mutagenic to animals as an alkylating agent through the oxidation of the furan ring, resulting in tumorigenesis. On the other hand, like benzofuran, dihydrobenzofurans have low toxicity to animals because of the absence of double bond (i.e., the oxidation site of the furan ring) in their chemical structures.

Table 1  Insect antifeedant activities of dihydrobenzofurans against *S. litura* and *P. xylostella* larvae.

<table>
<thead>
<tr>
<th></th>
<th><em>S. litura</em> ED₅₀ (μmol/cm²)</th>
<th><em>S. litura</em> ED₉₀ (μmol/cm²)</th>
<th><em>P. xylostella</em> ED₅₀ (μmol/cm²)</th>
<th><em>P. xylostella</em> ED₉₀ (μmol/cm²)</th>
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NT: Not Test

Fig. 3  Dihydrobenzofuran and furanobenzoquinone from *Cyperus* spp.

Fig. 4  Commercial insecticides included dihydrobenzofuran moiety in these structures.

4 CONCLUSION

A number of natural dihydrobenzofurans may be prepared using an electrochemical reaction. The SAR study using prepared dihydrobenzofurans indicated that these synthetic compounds also exhibit similar insect antifeedant activities to natural products. Search for lead compounds for pest control and subsequent SAR research will provide important findings about their drug development. However, their applications to actual crop protection require further evaluation of their phytotoxicity and environmental stability. The non-stilbene-type derivative (2) exhibited strong insect antifeedant activity against both species in this test. However, this dihydrobenzofuran inhibited the growth of lettuce (*Lactuca sativa*, cv. Great lakes) seedlings at 100 ppm. On the other hand, the stilbene-type derivative (11)
did not exhibit phytotoxicity, even at 500 ppm, in the same test. Additionally, non-stilbene-type derivatives easily undergo photo-oxidation, and stilbene-type derivatives are tolerant to photo-irradiation. Therefore, the stilbene-type derivative (11) represents a feasible lead for the development of insect control agents.

References

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