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Larvicidal and Adulticidal Activity of Alkylphthalide Derivatives from Rhizome of *Cnidium officinale* against *Drosophila melanogaster*

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The insecticidal activity of the chloroform extract of *Cnidium officinale* rhizomes and its constituents was investigated against larvae and adults of *Drosophila melanogaster* and compared with that of rotenone. Bioassay-guided isolation of the chloroform extract of *C. officinale* resulted in the isolation and characterization of four alkylphthalides, cnidilide (**1**), (*Z*)-ligustilide (**2**), (3*S*)-butylphthalide (**3**), and neocnidilide (**4**). The structures of these compounds were established by spectroscopic analysis. The isolated compounds **2**, **3**, and **4** exhibited LC_{50} values of 2.54, 4.99, and 9.90 μmol/mL of diet concentration against larvae of *D. melanogaster*, respectively. Against both sexes (males/females, 1:1) of adults (5−7 days old), compound **3** showed the most potent activity of the compounds isolated with the LD_{50} value of 5.93 μg/adult, comparable to that of rotenone (LD_{50} = 3.68 μg/adult). Structure−activity relationships of phthalides isolated suggest that the presence of conjugation with the carbonyl group in the lactone ring appeared to play an important role in the larvicidal activity. Acetylcholinesterase (prepared from the adult heads of *D. melanogaster*) inhibitory activity was also investigated *in vitro* to determine the insecticide mode of action for the acute adulticidal activity.

KEYWORDS: Natural insecticide; Apiaceae; *Cnidium officinale*; *Drosophila melanogaster*; alkylphthalide; structure−activity relationship; acetylcholinesterase inhibition

INTRODUCTION

Phthalides, natural volatile compounds present in apiaceous plants, have shown biological activities, such as anticholinergic, antispasmodic, smooth muscle relaxant, and centrally acting muscle relaxant effects (*1*−*4*). Phthalide derivatives have potential as a new natural pesticide because recent research indicates that a large number of phthalides have the insecticidal, herbicidal, nematicidal, antimicrobial, and acaricidal activities (*5*−*8*). In our previous work, two alkylphthalides along with two furanocoumarins were isolated as insecticidal principles of *Angelica acutiloba* KITAGAWA var. *sugiyamae* HIKINO (Apiaceae) against *Drosophila melanogaster* and (*Z*)-butylidenephthalide had the most potent activity against both larvae and adults (*9*).

In our search for new naturally occurring insecticidal phthalides, we investigated rhizomes of *Cnidium officinale* (Apiaceae), from which a wide variety of alkyl and hydroxyphthalides have been isolated (*10*−*12*). Anti-angiogenic and acetylcholinesterase inhibitory activities have been reported from *C. officinale* (*4*, *13*). The chloroform extract of *C. officinale* was found to exhibit larvicidal activity against *D. melanogaster*. Using *D. melanogaster* as a test insect is helpful in searching for insecticides of natural origin, which are often isolated in limited supply. Because of its small size, insecticidal activity can be detected using very small samples. Moreover, *D. melanogaster* has been used to examine insecticidal activity and insecticide mode of action because of its genetic accessibility (*15*). *C. officinale* is one of the most popular crude drugs for the treatment of obstetrical and gynecological disorders along with *A. acutiloba*, and is distributed in China and Japan. As for the chemical constituents, phthalide derivatives are specific for *C. officinale* and other apiaceous plants. Phthalides, which are components of the essential oils, are volatile, and the characteristic odor of Apiaceae plants is often due to the alkylphthalide derivatives. Monoterpenoids, components of the essential oils in many plants, are very important to insects because they can attract benefical insects, which can aid in pollination, and they can often help plants defend against harmful insects because of their toxicity and repellency. Although some terpenoids have been shown to possess insecticidal activity, the phthalides have not been investigated as such (*16*).

Plant essential oils, plant extracts, and their constituents can provide potential alternatives to synthetic insecticides that lead to insecticide resistance and environmental and human health concerns. Because of the worldwide attention toward pesticide

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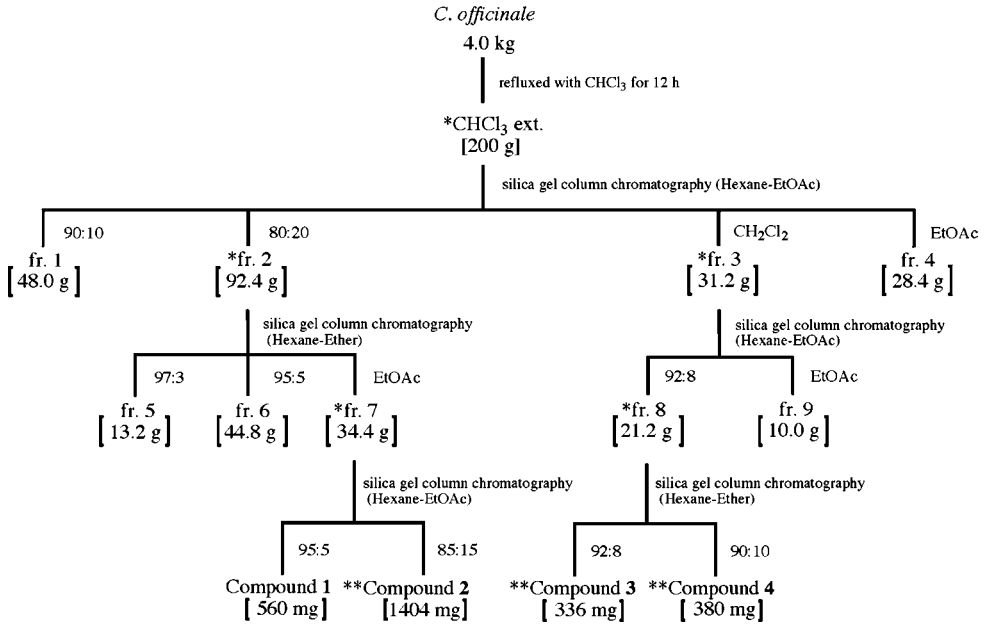

Figure 1. Isolation scheme for insecticidal compounds from *C. officinale* against larvae of *D. melanogaster* (*, active fraction; **, active compound).

residues in agricultural products, insecticides of natural origin are potentially useful for food safety (*17*). Although some naturally occurring compounds of plant origin have been reported as insecticides, most of them have not been fully studied yet.

Biological activity of *C. officinale* against insects has not been previously reported. In this paper, we investigated the bioassay-guided isolation and identification of active principles, structure−activity relationships in relation to their insecticidal activity against larvae and adults of *D. melanogaster*, and insecticide mode of action.

MATERIALS AND METHODS

Chemical Analysis. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra were recorded on a JEOL FX-500 NMR spectrometer. Tetramethylsilane (TMS) was used as the internal reference (δ 0.00) for ^{1}H NMR spectra measured in $CDCl_3$. This solvent was also used for ^{13}C NMR spectra. Electron impact mass spectra (EI−MS) were obtained at 70 eV by GC−MS on a Hewlett−Packard 5972 Series mass spectrometer interfaced with a Hewlett−Packard 5890 Gas chromatograph fitted with a column (HP-5MS, 30 m × 0.25 mm i.d., temperature of 140 °C, 4 °C/min). FAB−MS was obtained on a JEOL Tandem MStation JMS-700. IR spectra were determined with an FT/IR-470 Plus Fourier transform infrared spectrometer. Specific rotation was measured on a JASCO DIP-1000 digital polarimeter.

Plant Material. Commercially available air-dried rhizomes of *C. officinale* were obtained from Takasago Yakugyou Company (Osaka, Japan). 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB) was purchased from Tokyo Kasei Kougyou Company (Tokyo, Japan). Acetylthiocholine iodide (ATC) was purchased from Kanto Chemical Company (Tokyo, Japan). Rotenone (95% purity) was purchased from Sigma Chemical Company (St. Louis, MO).

Insects. *D. melanogaste*r used in bioassays was obtained from professor Ishikawa of the University of Tokyo (Japan). The colony of *D. melanogaster* has been maintained without exposure to any insecticides at 25 °C, RH > 60%, and 12:12 LD. Egg and larval stages take 12−36 h and 5−6 days, respectively, and adult longevity is about 60 days under the rearing conditions.

Extraction and Isolation of the Active Compounds. The isolation procedure from *C. officinale* is given in **Figure 1**. Air-dried rhizomes of *C. officinale* (4 kg) were extracted with chloroform under reflux for 12 h. The solvent was removed under reduced pressure to give a chloroform extract (200 g). This showed strong insecticidal activity

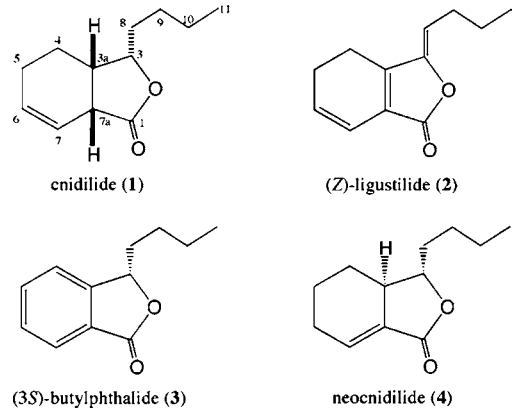

Figure 2. Structures of compounds **1**–**4** isolated from *C. officinale*.

(100% mortality at 2.0 mg/mL of diet) against larvae of *D. melanogaster*. To purify the active compounds, the extract was separated using bioassay-guided fractionation. The extract was fractionated by silica gel column chromatography (Merck 200 mesh) with hexane/ethyl acetate (EtOAc). The active fraction 2 eluting with hexane/EtOAc (80:20) was rechromatographed on silica gel with hexane/ether to give fractions 5−7. The active fraction 7 eluting with EtOAc was rechromatographed on silica gel with hexane/EtOAc (95:5) to give compound **1** (560 mg) and hexane/EtOAc (85:15) to give compound **2** (1404 mg). Another active fraction 3 was rechromatographed by silica gel with hexane/EtOAc to give fractions 8 and 9. The active fraction 8 eluting with hexane/EtOAc (92:8) was rechromatographed by silica gel with hexane/EtOAc (92:8) to give compound **3** (336 mg) and further eluted with hexane/EtOAc (90:10) to give compound **4** (380 mg). Compounds **1**−**4** were identified as cnidilide (**1**), (*Z*)-ligustilide (**2**), (3*S*)-butylphthalide (**3**), and neocnidilide (**4**) (**Figure 2**) by specific rotation, FAB−MS, EI−MS, IR, and ^{1}H and ^{13}C NMR, respectively.

Compound 1. Compound **1** was a colorless oil. $[\alpha]_D^{24.2}$ −117.0 ($CHCl_3$, *c* 0.6). FAB−MS (pos.) *m/z*: 195 $[M + H]^+$. EI−MS *m/z* (relative intensity %): 150 (16), 107 (9), 94 (30), 93 (38), 79 (100). IR (KBr, υ_{max}, cm^{-1}): 2929, 2857, 1770, 1175, 981, 968. ^{1}H NMR ($CDCl_3$) δ: 5.95 (1H, ddt, J = 10.0, 5.7, 2.0 Hz, H-6), 5.87 (1H, ddd, J = 10.0, 2.6, 4.6 Hz, H-7), 4.43 (1H, dd, J = 13.3, 5.2 Hz, H-3), 3.17 (1H, dddd, J = 2.4, 2.4, 4.6, 7.2 Hz, H-7a), 2.49 (1H, dddd, J = 13.3, 7.2, 4.6, 4.6 Hz, H-3a), 2.12−2.17 (1H, m, H-5), 1.94−2.03 (1H, m, H-5), 1.73−1.79 (2H, m, H-4,8), 1.55−1.62 (1H, m, H-8), 1.43−1.53 (1H, m, H-9), 1.35−1.43 (2H, m, H-9,10), 1.27−1.35 (1H, m, H-4), 0.93 (3H, t, J = 7.2 Hz, H-11). ^{13}C NMR ($CDCl_3$) δ: 176.7 (C-1),

130.3 (C-6), 121.0 (C-7), 81.7 (C-3), 42.4 (C-7a), 37.4 (C-3a), 29.1 (C-8), 28.1 (C-9), 23.4 (C-5), 22.5 (C-10), 19.3 (C-4), 13.9 (C-11). These spectral data were identical to the published data for cnidilide (*18*, *19*).

Compound 2. Compound **2** was isolated as an insecticidal principle from *Angelica acutiloba* in our previous work, and the spectral data of compound **2** was identical to the published data (*9*).

Compound 3. Compound **3** was a colorless oil. $[\alpha]_D^{27.7}$ −73.9 ($CHCl_3$, *c* 1.0). EI−MS *m*/*z* (relative intensity %): 190 (M^+, 2), 133 (100), 105 (28), 77 (13). IR (KBr, υ_{max}, cm^{-1}): 1763, 1615, 1467, 1285, 1062, 743, 694. ^1H NMR ($CDCl_3$) δ: 7.90 (1H, d, J = 7.5 Hz, H-7), 7.67 (1H, ddd, J = 7.6, 7.8, 0.9 Hz, H-5), 7.52 (1H,dd, J = 7.6, 7.5 Hz, H-6), 7.44 (1H, d, J = 7.8 Hz, H-4), 5.48 (1H, dd, J = 7.8, 4.0 Hz, H-3), 2.02−2.09 (1H, m, H-8), 1.73−1.81 (1H, m, H-8), 1.32−1.53 (4H, m, H-9,10), 0.91 (3H, t, J = 7.2 Hz, H-11). ^{13}C NMR ($CDCl_3$) δ: 170.6 (C-1), 150.1 (C-3a), 133.9 (C-5), 129.0 (C-6), 126.1(C-7a), 125.6 (C-7), 121.7 (C-4), 81.4 (C-3), 34.3 (C-8), 26.8 (C-9), 22.4 (C-10), 13.8 (C-11). These spectral data were identical to the published data of (3*S*)-butylphthalide (*20*, *21*).

Compound 4. Compound **4** was a colorless oil. $[\alpha]_D^{22.3}$ −92.8 ($CHCl_3$, *c* 0.6). EI−MS *m*/*z* (relative intensity %): 194 (M^+, 1), 137 (3), 108 (100), 80 (13), 79 (32). IR (KBr, υ_{max}, cm^{-1}): 2932, 2862, 1763, 1683, 1226, 1183, 1024, 726. ^1H NMR ($CDCl_3$) δ: 6.78 (1H, dt, J = 3.5, 3.5 Hz, H-7), 3.97 (1H, ddd, J = 8.9, 7.6, 5.2 Hz, H-3), 2.44−2.46 (1H, m, H-3a), 2.33 (1H, ddddd, J = 20.3, 6.8, 3.5, 3.7, 1.2 Hz, H-6), 2.15−2.25 (1H, m, H-6), 2.04−2.09 (1H, m, H-4), 1.94 (1H, dddd, J = 13.9, 6.8, 3.3, 3.2 Hz, H-5), 1.71−1.82 (2H, m, H-8), 1.48−1.58 (2H, m, H-5,9), 1.33−1.45 (2H, m, H-9,10), 1.13−1.22 (1H, m, H-4), 0.93 (3H, t, J = 7.2 Hz, H-11). ^{13}C NMR ($CDCl_3$) δ: 170.2 (C-1), 135.2 (C-7), 131.2 (C-7a), 85.3 (C-3), 43.1 (C-3a), 34.3 (C-8), 27.5 (C-9), 25.4 (C-4), 25.0 (C-6), 22.5 (C-10), 20.8 (C-5), 13.9 (C-11). These spectral data were identical to the published data of neocnidilide (*18*, *22*).

Bioassay for Larvicidal Activity of Test Compounds. The bioassay for larvicidal activity using *D. melanogaster* was carried out as follows (*9*). The LC_{50} values of the isolates and insecticide were determined based on six concentrations (16.0, 8.0, 4.0, 2.0, 1.0, and 0.5 μmol/mL of diet). Test compounds were dissolved in 50 μL of ethanol and mixed in 1 mL of artificial diet [brewer's yeast (60 g), glucose (80 g), agar (12 g), and propionic acid (8 mL) in water (1000 mL)]. A control diet was treated with 50 μL of ethanol. Treated and control (solvent only) insects were held at the same condition used for colony maintenance.

The artificial diet was poured into Petri dishes and placed on the bottom of the culture bottles to collect newly emerged eggs on the diet. About 100 adults from colonies of *D. melanogaster* were introduced into the culture bottle and allowed to oviposit for 3 h. After 3 h, the artificial diets in Petri dishes were removed, and 10 new eggs were collected from Petri dishes and transferred onto the diets containing the test compounds, insecticides, and solvent in 1.8 mL glass tubes and reared at 25 °C and RH > 90% for 8 days. The developmental stages were observed, and the numbers of pupae were recorded and compared with those of a control. A total of 10 new eggs were used in each of three replicates. LC_{50} is the lethal concentration for 50% mortality and was determined by log-probit analysis (*23*).

Bioassay for Acute Adulticidal Activity of Test Compounds. The acute adulticidal activity was determined by topical application on the abdomen of both sexes (males/females, 1:1) of adult (5−7 days old) *D. melanogaster* (*9*). Adults from culture bottles were iced to stop their movement and treated on their abdomens with each test compound at doses of 50, 10, 5, 1, and 0.5 μg/adult in 0.5 μL of acetone using a 10 μL microsyringe. Controls were treated with 0.5 μL of acetone. Treated and control (solvent only) insects were held at the same condition for colony maintenance. A total of 30 adults were used for each dose, and all doses were replicated 3 times. At 30 min after treatment, survival of adults was recorded. Mortality was defined as the inability to move, and we observed whether insects that showed inability to move recovered 3 h after treatment. LD_{50} is the lethal dose for 50% mortality and determined from log-probit analysis (*23*).

Inhibition of Acetylcholinesterase *in Vitro*. The method of Grundy and Still (*24*) was used to determine the acetylcholinesterase (AChE) inhibitory activity. An enzyme mixture containing AChE was extracted from excised heads of both sexes (males/females, 1:1) of adult (5−7 days old) *D. melanogaster*. About 1000 adults were frozen at −80 °C for 7 days. The frozen adults were shaken for 1 min with a mixer to detach their heads. Separation of the heads from bodies was then accomplished by sieving through mesh (40 mesh/cm^2) so as to allow only the heads to pass. The heads were then homogenized in 5 mL of 0.1 M phosphate buffer at pH 8.0. The crude homogenate was centrifuged at 14000*g* for 30 min, and the supernatant was used as the enzyme source. ATC was dissolved in 10 mL of 0.1 M phosphate buffer at pH 7.0, and 15 mg of $NaHCO_3$ was added.

Inhibition of AChE was determined according to the colorimetric method of Ellman et al. (*25*). Both the control and test solutions employed 0.2 mL of the enzyme solution and 0.1 mL of DTNB added to 2.4 mL of 0.1 M phosphate buffer (pH 8.0). The test solutions were added to each of the test compounds dissolved in 50 μL of ethanol. The control solution was similarly prepared by the addition of 50 μL of ethanol. Both control and each of the test solutions were preincubated at 25 °C for 5 min. After preincubation, the enzyme reaction was started by the addition of 40 μL of ATC followed by the incubation at 25 °C for 20 min. After 20 min, the absorbance at 412 nm was measured spectrophotometrically and compared with that of the control.

Table 1. Larvicidal Activity of Compounds **1**–**4** and Rotenone against *D. melanogaster* (on the Basis of the Number of Pupae)[a]

compound	LC_{50}[b] (μmol/mL of diet)	95% confidence limit	RT[c]
cnidilide (**1**)	>16.0	ND[d]	<0.001
(*Z*)-ligustilide (**2**)[e]	2.54 ± 0.19	1.95–3.55	0.009
(3*S*)-butylphthalide (**3**)	4.99 ± 0.52	3.86–7.09	0.004
neocnidilide (**4**)	9.90 ± 1.08	7.56–14.44	0.002
rotenone[e]	0.02 ± 0.01	0.02–0.03	1

[a] A total of 8 days after transferred eggs (10 eggs newly laid, 3 replicates). Test compounds of each concentration were dissolved in 50 μL of ethanol and mixed in 1 μL of artificial diet. [b] LC_{50} is the lethal concentration for 50% mortality, determined by log-probit analysis. LC_{50} values are the mean ± SE of three replicates. [c] Relative toxicity = LC_{50} value of rotenone/LC_{50} value of each test compound. [d] ND = not determined. [e] Data from ref *9*.

RESULTS

Isolation of Active Compounds. The chloroform extract of *C. officinale* was fractionated to identify insecticidal compounds by bioassay-guided isolation (**Figure 1**). Insecticidal compounds in the chloroform extract were isolated by SiO_2 column chromatography and identified as cnidilide (**1**), (*Z*)-ligustilide (**2**), (3*S*)-butylphthalide (**3**), and neocnidilide (**4**) by spectroscopic analysis.

Larvicidal Activity of Test Compounds. The larvicidal activity of compounds **1**−**4** against *D. melanogaster* is given in **Table 1**. Compounds **2**, **3**, and **4** showed larvicidal activity, and compound **2** had shown most insecticidal activity among the isolated compounds against larvae, with an LC_{50} value of 2.54 μmol/mL. Compounds **3** and **4** exhibited LC_{50} values of 4.99 and 9.90 μmol/mL, respectively. Cnidilide (**1**) was also active but at a higher concentration only. Compound **1** produced 43% mortality at 16.0 μmol/mL of diet, but an LC_{50} value was not determined in this range of concentrations. However, these isolated compounds were less active than rotenone (LC_{50} = 0.02 μmol/mL) (*9*). No mortality was observed in the controls.

Acute Adulticidal Activity of Test Compounds. The acute adulticidal activity of the isolated compounds was assessed by topical application on the abdomen of adults (**Table 2**). Compounds **1**, **3**, and **4** showed acute activity, and the most active compound was compound **3** with an LD_{50} value of 5.93 μg/adult. This indicated that compound **3** was comparable to

Table 2. Acute Adulticidal Activity of Compounds **1**–**4** and Rotenone against *D. melanogaster* (Expressed as Numbers Surviving)[a]

compound	LC_{50}[b] (μg/adult)	95% confidence limit	RT[c]
cnidilide (**1**)	9.17 ± 0.67	7.09–11.89	0.40
(*Z*)-ligustilide (**2**)[d]	>50.0	ND[e]	<0.07
(3*S*)-butylphthalide (**3**)	5.93 ± 0.54	4.45–8.01	0.62
neocnidilide (**4**)	10.82 ± 0.81	8.51–14.00	0.34
rotenone[d]	3.68 ± 0.14	3.33–4.06	1

[a] After 30 min, survival of adults was recorded (percent relative to the control). A total of 30 adults (males/females, 1:1, 5–7 days old) were tested for each dose, and all doses were replicated 3 times. Test compounds of each dose were dissolved in 0.5 μL of acetone and applied on the abdomen of adults with a 10 μL microsyringe. Negative controls were treated with 0.5 μL of acetone only. [b] LD_{50} is the lethal dose for 50% mortality, determined by log-probit analysis. LD_{50} values are the mean ± SE of three replicates. [c] Relative toxicity = LD_{50} value of rotenone/LD_{50} value of each test compound. [d] Data from ref *9*. [e] ND = not determined.

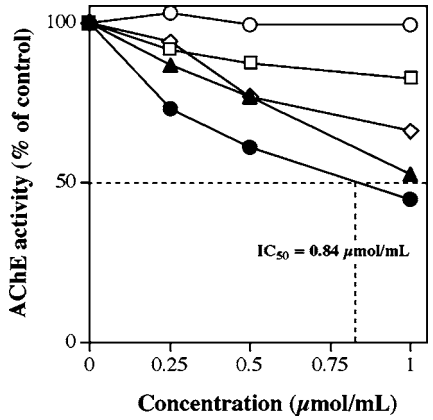

Figure 3. AChE inhibitory effect by compounds **1**–**4** and rotenone *in vitro*: cnidilide (**1**, □), (*Z*)-ligustilide (**2**, ◇), (3*S*)-butylphthalide (**3**, ▲), neocnidilide (**4**, ●), and rotenone (control, ○).

rotenone (LD_{50} = 3.68 μg/adult) (*9*). Both compounds **1** and **4** exhibited 97% mortality at a concentration of 50.0 μg/adult, with LD_{50} values of 9.17 and 10.82 μg/adult, respectively. However, no mortality was observed with compound **2** in this test. No insects that showed inability to move were observed, and there was no control mortality.

AChE Inhibitory Activity *in Vitro*. The *in vitro* study of AChE inhibitory activity by compounds **1**−**4** was examined to investigate the mode of action of acute adulticidal activity against adults of *D. melanogaster* (**Figure 3**). Compounds **3** and **4** gave comparable inhibition of AChE even though compound **3** was significantly more toxic in the acute bioassay. Compounds **1** and **2** had inhibitory activity, even though compounds **1** and **4** were equitoxic in the acute bioassay (*9*). As expected, rotenone had no activity because toxicity of rotenone to insects and mammals is attributable to the inhibition of NADH−ubiquinone oxidoreductase complex I activity (*9*).

DISCUSSION

Bioassay-guided isolation using larvae of *D. melanogaster* resulted in the isolation and characterization of larvicidal and adulticidal alkylphthalides, cnidilide (**1**), (*Z*)-ligustilide (**2**), (3*S*)-butylphthalide (**3**), and neocnidilide (**4**). Structure−activity relationships indicate that conjugation with the carbonyl group in the lactone ring appeared to play an important factor in the larvicidal activity: compounds **2**, **3**, and **4**, which have the conjugation with carbonyl group, were relatively more active than compound **1**. In addition to this indication, the numbers and position of the conjugation were important for activity: compound **2** (LC_{50} = 2.54 μmol/mL) was more active than compounds **3** (LC_{50} = 4.99 μmol/mL) or **4** (LC_{50} = 9.90 μmol/mL). (*Z*)-Butylidenephthalide, which has the aromatic ring and the double bond between the C-3 and C-8 position in the phthalide, exhibited an LC_{50} value of 0.94 μmol/mL in our previous work. Against adults, compounds **1**, **3**, and **4** were active. A structure−activity relationship indicated that aromaticity in the phthalide appeared to play an important role in activity. (*Z*)-Butylidenephthalide exhibited LD_{50} value of 0.84 μg/adult. Zheng et al. (*26*) reported that the five-membered lactone ring in phthalides is important for high glutathione *S*-transferase activity. Momin and Nair (*6*) reported that mosquitocidal, nematicidal, and antifungal compounds of *Apium graveolens* L. seeds were identified as three phthalides, sedanolide, senkyunolide-N, and senkyunolide-J. They suggested that the five-membered lactone ring along with the butyl side chain in phthalides might be important for biological activities. In addition to these reports, conjugation and aromaticity may play an important role for biological activity of the phthalides based on our results.

To investigate the insecticide mode of action, AChE inhibitory activity was assessed because some insecticides, including alkaloids (*27*), are known to inhibit AChE and phthalides have been isolated as anticholinergic substances from *A. acutiloba* (*1*). In our previous work, AChE activity was not inhibited by (*Z*)-butylidenephthalide, one of the most potent compounds against *D. melanogaster*. This was supported by the observation that butylidenephthalide acted as a fumigant and not a contact agent (*8*). However, (*Z*)-ligustilide (**2**), which was less active than (*Z*)-butylidenephthalide against both larvae and adults of *D. melanogaster*, slightly inhibited AChE activity. In the present study, compounds **3** and **4** were more active than compound **2**. Therefore, compounds **3** and **4** might have the same direct contact action, although AChE inhibitory activity dose not appear to be related to adulticidal activity. Some terpenoids are known as AChE inhibitors, and others are known as neurotoxic substances, such as antagonists of octopaminergic action (*28*, *29*). Therefore, octopaminergic action might be involved in the insecticidal activity of phthalides. However, an exact insecticide mode of action remains unknown.

As discussed above, *C. officinale* extracts and isolated alkylphthalides have the potential to be used as novel pest control agents. Although some monoterpenoids and other terpenes have been studied for their use as pesticides and repellents and a few of these compounds (*d*-limonene and linalool) are currently used against insects, phthalide derivatives have not been well-investigated. Plant extracts, essential oils, and their secondary constituents could be new tools for pest management because of their biological activity toward insects (*28*, *29*). Because these often biodegrade to nontoxic products, they could be much safer insect control agents. The Japanese government has established increasingly restrictive legislation regarding the maximum residue limits (MRLs) of pesticides in agricultural products (*16*). In Europe, organic production may use natural insecticides and not synthetic ones in pest control (*30*). Therefore, further discovery of insecticides of natural origin is needed. In conclusion, the insecticidal activity of *C. officinale* has been evaluated. Four active principles were isolated as potential natural insecticides. This is the first report of the larvicidal and adulticidal activity of *C. officinale* and compounds **1**, **3**, and **4**. Further studies on the insecticidal mechanisms are required.

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