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Insecticidal Effect and Chemical Composition of the Volatile Oil from *Bergenia ligulata*

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ABSTRACT: The chemical composition of the volatile oil from roots of *Bergenia ligulalta* was analyzed by GC-MS. A total of 97 compounds were identified. (+)-(6*S*)-Parasorbic acid (**1**) (47.45%), isovaleric acid (6.25%), 1,8-cineole (4.24%), (*Z*)-asarone (3.50%), and terpinen-4-ol (2.96%) were the most prominent constituents. (+)-(6*S*)-Parasorbic acid (**1**) was isolated and characterized by spectroscopic data. This is the first report of the existence of (+)-(6*S*)-parasorbic acid in the saxifrage family. The volatile oil and the isolated compound were tested against *Drosophila melanogaster*. The results obtained showed that the volatile oil from roots could be considered as natural insecticidal effect agents.

KEYWORDS: *Bergenia ligulata*, GC-MS, (+)-(6*S*)-parasorbic acid (**1**), *Drosophila melanogaster*, insecticidal activitiy, acetylcholinesterase inhibition

INTRODUCTION

Plants can provide potential alternatives to the currently used insecticides that seem to cause insecticide resistance and environmental and human health concerns; because they constitute a rich source of bioactive chemicals, such as terpenoids, alkaloids, and flavonoids, against insects, they have evolved strategies to interact with other organisms for self-defense.[1] In particular, monoterpenoids, components of volatile oils in many plants, are very important to plants because they can attract beneficial insects, which can aid in pollination, and they can help plants defend against harmful insects because of their high influence of volatility.[2] These often biodegrade to nontoxic products, so they could be much safer insect control agents and more suitable for use in integrated pest management (IPM).[3] Because of the worldwide attention toward pesticide residues in agricultural products, insecticides of natural origin are very important in food safety.[4] Moreover, these compounds also can be good leads for more effective insecticides such as pyrethroids, which have been focused on by many workers due to their strong insecticidal activity with safe use for mammals and plants.[5] Although some naturally occurring compounds have been identified as insecticides, antifeedants, attractants, and repellents, such as azadirachtin from *Azadirachta indica*, which is the most important and potent insect antifeedant in use over recent years, most of them have not been fully studied yet.[6]

In our search for new naturally occurring insecticidal compounds, we used crude drugs with a history of safe use as medicines. In the course of screening for novel naturally occurring insecticides, the chloroform extract of the roots of *Angelica acutiloba* Kitagawa var. *sugiyamae* Hikino (Apiaceae) was found to exhibit insecticidal activity against larvae of *Drosophila melanogaster*. Using *D. melanogaster* as a test insect is helpful in searching for insecticides of natural origin, which often have a limited sample; due to its small size, the insecticidal activity can be detected with very little sample.

Moreover, *D. melanogaster* has been used to examine the insecticidal activity and insecticide mode of action because of its genetic accessibility.[7]

Bergenia ligulata (syn. *Saxifraga*) is perennial herb that grows wild in northern India particularly between stones and rocks. It is called "Pashanbheda" in India. The diameter of rhizomes is about 1 cm, the surface is brown and coarse, and the inner skin is smooth.[8] This plant has been used in Ayurvedic formulations for various ailments. The rhizomes of *B. ligulata* have diuretic, tonic, astringent, laxative, and other activities.[9−11] Chemical components of *B. ligulata* indicated the presence of bergenin, β-sitosterol, β-sitosterol-D-glucoside,[12] a number of phenolic compounds such as (+)-afzelechin[13] and catechin, and other compounds.[14,15] Recently, the inhibiton of α-glucosidase by a methanol−water extract of this plant exhibiting antiviral activity was reported[16] as well as an inhibitory effect on influenza virus A.[17] It is thought that the volatile components constitute the causative agent of these biological activities. *B. ligulata* has a charactaristic sweet and woody-like odor. Monoterpenoids and other volatile compounds have been shown to possess insecticidal and various activities against insects, but *B. ligulata* has not been understood well.

The purpose of this work is to present the chemical composition of the volatile oil from roots of *B. ligulata*, as well as its insectidal activity against *D. melanogaster*, insecticidal mode of action.

MATERIALS AND METHODS

Plant. Roots of *B. ligulata* were provided by Koei Kogyo Co., Ltd. (Tokyo, Japan).

Gas Chromatography−Mass Spectorometry (GC-MS) Conditions. GC-MS used an Agilent 6890-5973 instrument. The sample was analyzed on a fused-sillica capillary column HP-5MS

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Table 1. Components of the Volatile Oil from *Bergenin ligulata*

no.	RI[a] RI-5	RI-W	compound[b]	wt %	ID method[c]	reference source[d]
1	773	1268	pentanol	tr	RI, MS	Wako
2	799	1090	hexanal	0.32	RI, MS	Wako
3	812	892	ethyl acetate	0.15	RI, MS	Wako
4	829	1460	furfural	0.18	RI, MS	Wako
5	835		2-(methylthio)ethanol	1.3	RI, MS	Wako
6	875	1687	isovaleric acid	6.25	RI, MS	Wako
7	883		santene	tr	RI, MS	other
8	890	1260	styrene	tr	RI, MS	Wako
9	901	1372	hexanol	tr	RI, MS	Wako
10	932	1021	α-pinene	0.24	RI, MS	Wako
11	959	1520	benzaidehyde	0.54	RI, MS	Wako
12	969	1443	heptanol	0.46	RI, MS	Wako
13	973	1107	sabinene	1.10	RI, MS	other
14	976	1092	β-pinene	0.10	RI, MS	Wako
15	987		6-methyl-5-hepten-2-one	tr	RI, MS	Wako
16	991		2-pentylfuran	0.36	RI, MS	Wako
17	1003	1846	hexanoic acid	0.75	RI, MS	Wako
18	1017	1161	α-terpinene	0.23	RI, MS	Wako
19	1024	1283	*p*-cymene	0.18	RI, MS	Wako
20	1029	1195	limonene	0.28	RI, MS	Aldrich
21	1032	1204	1,8-cineole	4.24	RI, MS	Wako
22	1036	1891	benzyl alcohol	0.31	RI, MS	Wako
23	1044		phenyl acetaldehyde	0.29	RI, MS	Wako
24	1059	1224	γ-terpinene	0.49	RI, MS	Wako
25	1066	1644	acetophenone	0.14	RI, MS	Wako
26	1085	1787	(+)-(6*S*)-parasorbic acid[e]	47.45		
27	1088		*p*-mentha-2,4(8)-diene	tr	RI, MS	other
28	1089		linalool oxide	0.66	RI, MS	Aldrich
29	1093	1882	guaiacol	tr	RI, MS	Wako
30	1094		6-camphenone	tr	RI, MS	other
31	1097	1772	δ-hexalactone	0.52	RI, MS	Wako
32	1102	1540	linalool	0.64	RI, MS	Wako
33	1105	1390	nonanal	0.42	RI, MS	Wako
34	1115	1810	phenylethanol	0.32	RI, MS	Wako
35	1122	1595	isophorone	0.37	RI, MS	Wako
36	1141	1630	*cis-p*-menth-2-en-1 -ol	tr	RI, MS	other
37	1147	1545	camphor	0.65	RI, MS	Aldrich
38	1148		veratrole	0.31	RI, MS	Wako
39	1160	1714	(2*E*)-nonen-1-al	0.26	RI, MS	Wako
40	1165	1543	pinocarvone	tr	RI, MS	other
41	1168	1706	borneol	1.37	RI, MS	Wako
42	1175	1636	menthol	0.83	RI.MS	Aldrich
43	1180	1608	terpinen-4-ol	2.96	RI, MS	Wako
44	1185	1818	*p*-cymen-8-ol	1.43	RI, MS	other
45	1193	1675	α-terpineol	1.2	RI, MS	other
46	1196		methyl salicylate	0.32	RI, MS	Wako
47	1199	1792	myrtenol	tr	RI, MS	Wako
48	1206	1729	dodecanai	tr	RI, MS	Wako
49	1213		octanol acetate	0.55	RI, MS	Wako
50	1242	1789	cumin aldehyde	0.3	RI, MS	Wako
51	1246	1718	carvone	0.32	RI, MS	other
52	1251		perilla ketone	0.36	RI, MS	other
53	1257	1705	piperitone	tr	RI, MS	other
54	1257	1850	geraniol	tr	RI, MS	Wako
55	1273		2,4,6-trimethylphenol	0.6	RI, MS	Wako
56	1277	1788	perilla aldehyde	0.46	RI, MS	other
57	1279	2165	nonanoic acid	tr	RI, MS	Wako
58	1288	1820	(*E*)-anethole	0.27	RI, MS	Aldrich
59	1295	2153	thymol	0.34	RI, MS	Wako
60	1304	2229	carvacrol	tr	RI, MS	Wako
61	1316	2200	4-vinylguaiacol	0.84	RI, MS	Wako
62	1318	1779	(2*E*,4*Z*)-decadienal	0.47	RI, MS	other
63	1365	2018	γ-nonalactone	tr	RI, MS	Wako
64	1370	1481	isoledene	tr	RI, MS	Aldrich
65	1376	1485	β-patchoulene	0.24	RI, MS	other
66	1448		seychellene	tr	RI, MS	other
67	1455	1863	**geranyl acetone**	tr	RI, MS	Wako
68	1459		(*Z*)-methylisoeugenol	tr	RI, MS	other
69	1460	1647	α-humulene	tr	RI, MS	Aldrich
70	1461		neryl propionate	tr	RI, MS	other
71	1463		dihydroaromadendrane	tr	RI, MS	other
72	1481		massoia lactone	tr	RI, MS	other
73	1487	1777	*ar*-curcumene	0.69	RI, MS	other
74	1505	1706	muurolene	tr	RI, MS	other
75	1511	1613	α-bulnesene	0.19	RI, MS	Aldrich
76	1515	1947	β-ionone	0.26	RI, MS	Aldrich
77	1528	1844	*trans*-calamenene	0.33	RI, MS	other
78	1534		kessane	0.47	RI, MS	other
79	1549	1916	α-calacorene	0.33	RI, MS	other
80	1554	2058	elemol	0.23	RI, MS	other
81	1564		1,3,5-trimethylnaphthalene	tr	RI, MS	other
82	1577		γ-asarone	tr	RI, MS	other
83	1583		*ar*-turmerol	0.44	RI, MS[f]	
84	1598		*ar*-dihydroturmerone	0.38	RI, MS[f]	
85	1616	2071	humulene epoxide II	0.31	RI, MS	other
86	1625	2318	(*Z*)-asarone	3.5	RI, MS	Aldrich
87	1638	2188	γ-eudesmol	0.83	RI, MS	other
88	1647	2186	α-muurolol	1.00	RI, MS	other
89	1658	2242	β-eudesmol	1.02	RI, MS	Wako
90	1667	2156	patchouli alcohol	1.29	RI, MS	other
91	1670		*ar*-turmerone	1.77	RI, MS[f]	
92	1678	2450	(3*Z*)-butylidene phthalide	tr	RI, MS	other
93	1681	2233	cadalene	0.25	RI, MS	other
94	1684	2445	(*E*)-asarone	0.51	RI, MS	Wako
95	1707		9-methyl-9*H*-fluorene	0.28	RI, MS	other
96	1780		phenanthrene	tr	RI, MS	Wako
97	1973	2842	palmitic acid	1.81	RI, MS	Wako
			total	98.26		

[a] Compounds are listed in the order of their elution time from HP-5MS and DB-WAX columns. The presence of compound is indicated by its GC-FID percentage. [b] RI, retention indices determined on HP-5MS and DB-WAX columns, using the homologous series of *n*-alkanes (C8−C27). [c] Identification methods: RI, retention index; MS, mass spectrum. [d] Reference materials were obtained from commercial source and our previous studies: Wako, Wako Pure Chemical Industries Ltd. (Osaka, Japan); Aldrich, Sigma-Aldrich, St. Louis, MO; other, reference mass spectrum and retention index were measured outside the author's laboratory (the identification is therefore considered to be tentative). [e] (+)-(6*S*)-Parasorbic acid was isolated, and it was identified with NMR, EI-MS,and IR.[32] [f] Our previous studies (refs 83, 84, and 91 from Fujiwara et al.[33]

(polydimethylsiloxane, 30 m × 0.25 mm i.d., film thickness = 0.25 μm) and DB-WAX (15 m × 0.25 mm i.d., film thickness 0.25 μm). The oven temperature was programmed from 40 to 260 °C at a rate of 4 °C/min and held at 260 °C for 5 min. The flow rate of carrier gas (He) was 1.8 mL/min. The injector and detector temperarures were 270 and 280 °C, respectively, with the actual temperature in the MS source reaching approximately 230 °C, and the ionization energy was 70 eV. The mass range was 39−450 amu. After 6 mg of oil had been diluted with 500 μL of diethyl ether, 1 μL of the dilution was injected, and the split ratio was 1:10.

Reference Materials. Reference materials of mass spectra and GC retention indices were obtained from commercial sources and our previous studies (see Table 1 for details).

Identification of Constituents. Identification of the volatile oil components was carried out by comparison of their relative retention times with those of authentic samples or by comparison of their retention indices (RI) relative to the series of *n*-hydrocarbons. Computer matching against commercial (NIST 98, Mass Finder 3.1 and Aroma Office)[18,19] and homemade library mass spectra made up of pure substances and components of known oils and MS literature data was also used for identification.

Insects. *D. melanogaster* used in bioassays for insecticidal activities against adults was distributed from Professor Ishikawa of the University of Tokyo. The colony of *D. melanogaster* has been maintained without exposure to any insecticides and insects at 25 °C, RH > 60%, and 12 h light/12 h dark cycle. Egg and larval periods are 12−36 h and 5−6 days, respectively, and adult longevity is about 60 days in the rearing condition.

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 adults (5−7 days old) of *D. melanogaster*.[20] Adults from culture bottles were iced to stop their movement and treated on their abdomens with each of the test compounds at doses of 20, 2, and 0.2 μg/adult in 0.5 μL of acetone with a 10 μL microsyringe. Controls were treated with 0.5 μL of acetone. Samples treated and control (solvent only) insects were held at the same conditions for colony maintenance. Thirty adults were used for each dose, and all doses were replicated three times. Three hours after treatment, survival of adults was recorded. Mortality was defined as inability to move and ensured whether insects that showed inability to move recovered. LD_{50} is the lethal dose for 50% mortality and determined from log-probit analysis.[21]

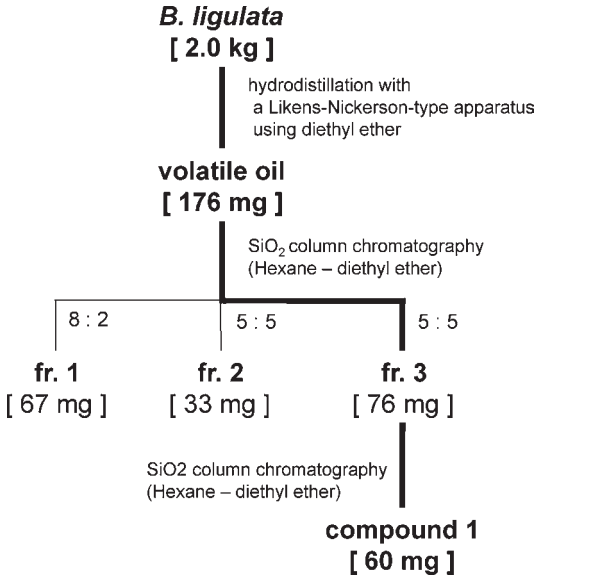

Figure 1. Isolation scheme of compound **1** from the volatile oil of roots of *B. ligurata*.

Inhibition of Acetylcholinesterase (AChE) in Vitro. The method of Grundy and Still[22] was used to determine the 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 the 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.[23] 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 at 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. The control and each of the test solutions was preincubated at 25 °C for 5 min. After preincubation, the enzyme reaction was started by the addition of 40 μL of ATC followed by 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.

Isolation of the Volatile Oil. Two kilograms of tips of dry roots from *B. ligulata* was hydrodistilled with a Likens−Nickerson-type apparatus using diethyl ether to yield 0.0088% of yellow oil, which was dried over anhydrous sodium sulfate prior to analyses.

Isolation of (+)-(6*S*)-Parasorbic Acid. Compound **1** was extracted and isolated from *B. ligulata* by silica gel column chromatography (Figure 1). Two kilograms of tips of dry roots was hydrodistilled with a Likens-Nickerson-type apparatus using diethyl ether to yield 176 mg of yellow oil, which was dried over anhydrous sodium sulfate prior to analyses. The volatile oil was fractionated by silica gel column chromatography (200 mesh) with hexane and diethyl ether as eluants; thereby compound **1** (60 mg) was isolated from fraction 3. Compound **1** was identified as (+)-(6*S*)-parasorbic acid by NMR and EI-MS spectroscopy.

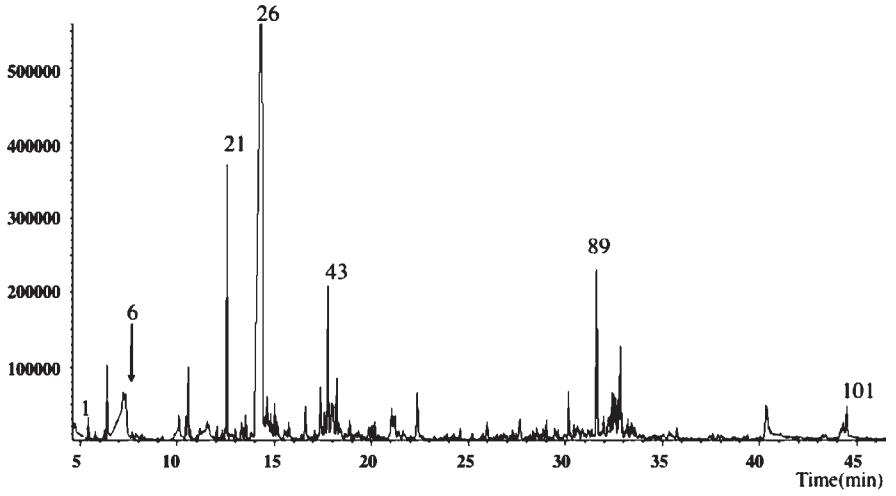

Figure 2. Gas chromatogram of the volatile oil from roots of *B. ligulata*.

(+)-(6*S*)-parasorbic acid (**1**): $[\alpha]^{22}_{D}$ +180.8° ($CHCl_3$, *c* 0.3); EI-MS, *m*/*z* (rel intensity) 112 ($[M]^+$, 3) 97 (9), 68 (100), 39 (17), 223 (37); IR (KBr) υ_{max} cm^{-1}, 2982, 2937, 1725, 1390, 1247, 1054; ^1H NMR ($CDCl_3$) δ 1.45 (3H, d, *J* = 6.5 Hz, H-7), 2.31 (1H, ddt, *J* = 18.5, 11.0, 2.5 Hz, H-5a), 2.38 (1H, dddd, *J* = 18.5, 6.0, 4.5, 1.0 Hz, H-5b), 4.58 (1H, m, H-6), 6.03 (1H, ddd, *J* = 10.0, 2.5, 1.0 Hz, H-3), 6.88 (1H, ddd, *J* = 10.0, 6.0, 2.5 Hz, H-4); ^{13}C NMR δ 20.7 (C-7), 31.0 (C-5), 74.3 (C-6), 121.3 (C-3), 144.9 (C-4), 164.5 (C-2).

RESULTS AND DISCUSSION

Table 1 shows the identified constituents and their weight percentage composition, as well as their retention index (RI) values listed in order of elution from the HP-5MS capillary column. In the volatile oil, 97 components accounting for 98.26% of the total oil were identified. The volatile oil from *B. ligulata* was analyzed on GC-MS, and the gas chromatogram is shown in Figure 2. As can be noted from Figure 3, the main components were (+)-(6*S*)-parasorbic acid (47.45%), isovaleric acid (6.25%), 1,8-cineole (4.24%), (*Z*)-asarone (3.50%), and terpinen-4-ol (2.96%). In the preliminary analysis of the oil from *B. ligulata* (Figure 2), the peak corresponding to (+)-(6*S*)-parasorbic acid (**1**) (t_R 14.29 min) was not identified from its retention index and mass spectraum. For this reason, an aliquot of the volatile oil from *B. ligulata* was subjected to silica gel chromatography, and compound **1**, in the pure form, was isolated and characterized by NMR spectral data. Because (+)-(6*S*)-parasorbic acid (**1**) is known to exhibit inhibitory activity against a variety of plants,[24,25] it may well be responsible for the allelochemical activity in *B. ligulata* and, at the same time, offer some protection to the plant from fungal infection.

The acute toxicity of (+)-(6*S*)-parasorbic acid (**1**) and volatile oil from *B. ligulata* is shown in Table 2. Against adults, (+)-(6*S*)-parasorbic acid (**1**) and volatile oil from *B. ligulata* were active; (+)-(6*S*)-parasorbic acid (**1**) was more insecticidally active than the volatile oil (Table 2).

The major finding of this study is the potent insecticidal activity of volatile (+)-(6*S*)-parasorbic acid (**1**) against adults of *D. melanogaster* because compound **1** was more active than rotenone. The volatile oil of *B. ligulata* was less active toward adults than compound **1**. This result indicates that the activity is caused by synergy with other compounds. We previously reported that (−)-7-epi-deoxynupharidine, (−)-tetrahydroberberine, and (−)-canadine were isolated as active ingredients against *D. melanogaster* with LD_{50} values of 0.9, 2.5, and 2.5 μg/adult, respectively.[20,26,27] Compared with the insecticidal activities toward adults of *D. melanogaster* in our previous related works, (+)-(6*S*)-parasorbic acid (**1**) has one of the most potent activities. Moreover, conjugation with the carbonyl group in the lactone ring appeared to play an important factor in the insecticidal activity in previously works.[28,30] In addition to these reports, conjugation with the carbonyl group in the lactone ring may play an important role for insecticidal activity of (+)-(6*S*)-parasorbic acid.

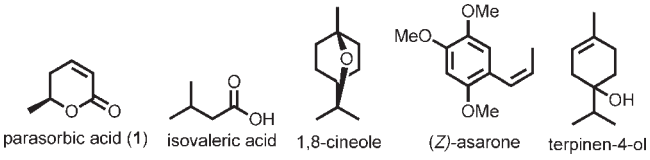

Figure 3. Structures of the main components of the volatile oil from roots of *B. ligulata*.

To investigate the insecticide mode of action, AChE inhibitory activity was assessed. The in vitro study of AChE inhibitory activity by (+)-(6*S*)-parasorbic acid (**1**) was examined to clarify the mode of action of acute adulticidal activity against *D. melanogaster* adults (Figure 4). AChE activity was not inhibited by (+)-(6*S*)-parasorbic acid (**1**). Rotenone had no activity because the acute toxicities of rotenone to insects and mammals are attributable to the inhibition of NADH-ubiquinone oxidoreductase complex I activity.[29]

In our previous work, AChE activity was not inhibited by (*Z*)-butylidene phthalide, one of the most potent compounds against *D. melanogaster*.[30] This was supported by the observation that butylidene phthalide acted as a fumigant and not as a contact agent.[31] In this study, (+)-(6*S*)-parasorbic acid (**1**) did not inhibit AChE activity. Therefore, one possibility is that

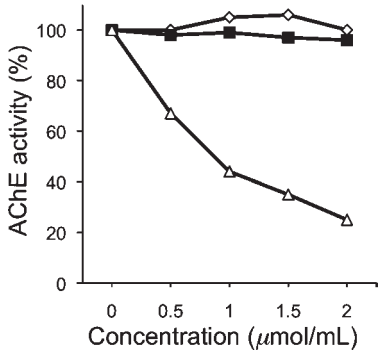

Figure 4. AChE inhibitory activity by (+)-(6*S*)-parasorbic acid (**1**), (+)-pulegone, and rotenone (control) in vitro: (+)-(6*S*)-parasorbic acid (**1**, ■); (+)-pulegone (positive control, △); rotenone (control, ◇).

Table 2. Adulticidal Activity of Volatile Oil, Compound 1, and Rotenone against *D. melanogaster* (Expressed as Numbers of Survival)[a]

		dose[b] (ng/adult)										
compound	control	20	15.0	10.0	5.0	2.0	1.5	1.0	0.5	LD_{50}[c] (μg/adult)	95% confidence limit	RT[d]
volatile oil	10, 10, 10	0, 0, 0	0, 0, 0	7, 6, 7	5, 3, 5	10, 10, 10	ND[e]	ND	ND	7.11	6.17−8.09	0.52
1		0, 0, 0		ND	ND	0, 0, 0	5, 5, 3	9, 10, 10	10, 10, 10	1.44	1.33−1.54	2.56
rotenone										3.68		1.00

[a] After 3 h, survival of adults was recorded (percent relative to control). Thirty adults {males/females 1:1, 5−7 days old) were tested for each dose, and all doses were replicated three times. [b] 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. [c] LD_{50} is the lethal dose for 50% mortality, determined by logprobit analysis. [d] Relative toxicity = LD_{50} value of each test compounds. [e] ND = not determined.

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(+)-(6*S*)-parasorbic acid (**1**) acts as a fumigant and not a direct contact agent. However, an exact insecticide mode of action remains unknown.

Consequently, this result suggests that plants containing a high amount of (+)-(6*S*)-parasorbic acid (**1**) are highly affective against insects, and one of the roles of this compound might be to prevent attack from harmful insects for plants. Although the activities of monoterpenoids and other terpenoids have been studied by many workers and a few of these compounds are currently used commercially as pesticides or repellents (limonene, menthol, linalool),[2] (+)-(6*S*)-parasorbic acid (**1**) has not been investigated well. The result of this study proposed that (+)-(6*S*)-parasorbic acid (**1**) and plants containing it might be used as a new tool for the protection of plants from harmful insects in organic agriculture. The Japanese government has established increasingly restrictive legislation regarding the maximum residue limits (MRLs) of pesticides in agricultural products.[4] In Europe, organic production may use natural insecticides and not synthetic ones in pest control.[29] It is concluded that further studies about insecticides of natural origin are essential to find new plants and compounds with insecticidal effects and to find a new strategy of plant protection with environmental safety.

In conclusion, (+)-(6*S*)-parasorbic acid (**1**) and volatile oil from roots of *B. ligulata* and its active compounds have been evaluated as potential natural insecticides using bioassay against adults of *D. melanogaster*. Further studies of the mechanism of insects and mammals in vitro and in vivo are needed to understand the pharmacological actions of (+)-(6*S*)-parasorbic acid (**1**). This is the first report on the insecticidal activity of (+)-(6*S*)-parasorbic acid (**1**) and volatile oil of *B. ligulata* against insects. We hope that this result will be used in the future to obtain safe foods for agriculture.

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