Structure-Activity Relationship of Acetylenes from Galls of *Hedera rhombea* as Plant Growth Inhibitors

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The structure-activity relationship of 12 isolated acetylenes from galls of *Hedera rhombea* (Araliaceae) induced by *Asphondylia* sp. (Cecidomyiidae) and their derivatives has been studied for the inhibition of the shoot and root growth of rice, perennial ryegrass, cockscomb, lettuce, and cress. Almost all acetylenes generally showed growth inhibitory activity. The diacetylenes exhibited higher activity than the monoacetylenes, suggesting that a conjugated diyne segment is essential for the activity. On the other hand, the acetylenes with a nonoxidated methylene group at C-8 showed stronger activity comparing with those possessing hydroxy and acetoxy groups at C-8. Furthermore, it has been demonstrated that the acetylenes bearing a terminal olefinic group at C-16,C-17 enhanced the activity. It is thus clarified that important sites for the activity of the acetylenes from galls of *H. rhombea* are a conjugated diyne and a terminal olefinic group connecting to the aliphatic chain and that less oxidated compounds show more activity.

Key words: Hedera rhombea, Acetylenes, Structure-Activity Relationship

Introduction

Acetylenes have been found in many families of higher plants, such as Araliaceae, Campanulaceae, Compositae, Olacaceae, Pottosporaceae, Santalaceae, and Umbelliferae (Christensen and Lam, 1991; Christensen, 1992; Kraus *et al.*, 1998). It has been reported that the potent allelopathic C_{10}-polyacetylene, *cis*-dehydromatricaria ester (*cis*-DME) from *Solidago altissima* L. and the C_{17}-polyacetylene, falcarindiol from *Glehnia littoralis* F. Schm. exhibit plant growth inhibitory activities (Kobayashi *et al.*, 1980; Ito *et al.*, 1998; Satoh *et al.*, 1996). However, to the best of our knowledge, effects on plant growth of other C_{17}-acetylene series, structurely related to falcarindiol, have not been studied. In this context, bioactivities of isolated C_{17}-acetylenes from galls of *Hedera rhombea* and their derivatives on plant growth were investigated. In this paper, the structure-activity relationship of the C_{17}-acetylenes was studied to reveal the chemical structure required for the plant inhibitory activity.

Results and Discussion

Twelve known acetylenic compounds, 8-acetoxyfalcarinol (**1**), falcarinol (**2**), ginsenoyne J (**3**), dehydrofalcarindiol (**4**), falcarindiol (**5**), crithmumdiol (**6**), PQ-2 (**7**), panaxydol (**8**), PQ-6 (**9**), dehydrofalcarindiol-8-acetate (8-acetoxydehydrofalcarinol) (**10**), didehydrofalcarinol (**11**), and 11,12-dehydrofalcarinol (**12**) (Fig. 1) were isolated from MeOH extracts of galls of *H. rhombea* and they were identified by comparison of their spectroscopic data with literature values (Bohlmann and Zdero, 1975; Otsuka *et al.*, 1981; Hirakura *et al.*, 1992; Tanaka *et al.*, 1977; Setzer *et al.*, 1995; Ruberto and Amico, 1999; Fujimoto *et al.*, 1991, 1992; Ahn and Kim, 1988; Bohlmann and Fritz, 1979; Hausen *et al.*, 1987; Gafner and Rodriguez, 1989). Oxidation of **2** with active MnO_2 gave falcarinone (**13**) (Schule and Potter, 1977), and 1,2,9,10-diepoxyheptadeca-4,6-diyne-3,8-diol (**14**) was obtained from **5** by epoxidation with *m*CPBA.

The structure-activity relationship of the C_{17}-acetylenes **1**–**12** from galls of *H. rhombea* and of their derivatives **13** and **14** has been studied for the inhibition of the shoot and root growth of monocotyledonous (rice and perennial ryegrass) and dicotyledonous plants (cockscomb, lettuce, and cress) in each test solution of 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} M. The results at the concentration 10^{-4}, 10^{-5}, and 10^{-6} M are shown in Table I since there was little difference among acetylenes **1**–**14** at 10^{-7} M. They generally showed growth inhibitory

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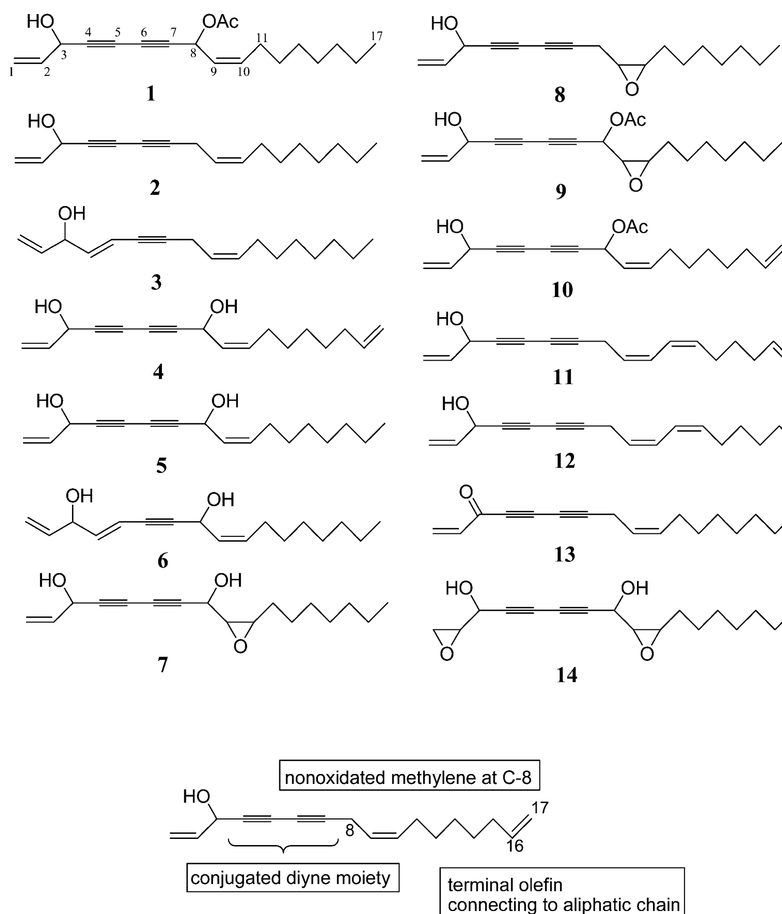

Fig. 1. Structures of 8-acetoxyfalcarinol (**1**), falcarinol (**2**), ginsenoyne J (**3**), dehydrofalcarindiol (**4**), falcarindiol (**5**), crithmumdiol (**6**), PQ-2 (**7**), panaxydol (**8**), PQ-6 (**9**), dehydrofalcarindiol-8-acetate (8-acetoxydehydrofalcarinol) (**10**), didehydrofalcarinol (**11**), 11,12-dehydrofalcarinol (**12**), falcarinone (**13**), 1,2,9,10-diepoxyheptadeca-4,6-diyne-3,8-diol (**14**) and structural requirement for growth-inhibitory activity.

activity against both monocotyledonous and dicotyledonous plants, although against lettuce most of the acetylenes manifest less or no activity on the shoots and roots. Among these acetylenes, the highest active compounds appeared to be **2**, **4**, and **13**. Diacetylenes such as **2** and **5** showed stronger activity than the corresponding monoacetylenes (**3** and **6**), suggesting that a conjugated diyne moiety is essential for activity. Meanwhile, it has been shown that compounds **2**, **3**, and **8**, which have a nonoxidated methylene group at C-8, exhibit higher activity comparing with those containing a hydroxy group at C-8 (**4**, **6**, and **7**) or that with an acetoxy group at the same position (**1** and **9**). However, compounds **11** and **12** with a double bond at C-11,C-12 showed quite weak activity in spite of their bearing nonoxidated methylene group at C-8, implying that the presence of a conjugated double bond at C-9,C-10 and C-11,C-12 reduced the activity. Furthermore, polyacetylenes (**4**, **10**, and **11**) possessing a terminal olefinic group at C-16,C-17 showed stronger activity than compounds **5**, **1**, and **12**, which do not have a terminal olefinic group at this position. Among the polyacetylenes (**7**, **8**, **9**, and **14**) containing an epoxide ring, the activity of **14** with two epoxide rings seemed to be stronger than that of the monoepoxides (**7**, **8**, and **9**).

These results suggested that the important sites for the activity of a series of polyacetylenes from

Table I. Inhibitory activity of C_{17}-acetylenes from galls of *H. rhombea* on the growth of roots and shoots of test plants.

Compound	% of control (root shoot)					
	10^{-4} M		10^{-5} M		10^{-6} M	
1	78 ± 1	64 ± 3	89 ± 3	72 ± 3	93 ± 2	96 ± 1
	97 ± 6	100 ± 4	100 ± 3	101 ± 4	91 ± 2	99 ± 4
	75 ± 7	57 ± 3	95 ± 4	65 ± 3	101 ± 5	84 ± 2
	73 ± 4	74 ± 4	90 ± 4	84 ± 6	90 ± 4	88 ± 4
	84 ± 1	96 ± 4	98 ± 5	96 ± 6	101 ± 4	95 ± 3
2	31 ± 2	40 ± 2	47 ± 2	67 ± 2	74 ± 8	86 ± 7
	73 ± 5	45 ± 2	75 ± 5	48 ± 4	99 ± 5	62 ± 4
	38 ± 2	32 ± 3	49 ± 4	51 ± 3	67 ± 5	68 ± 2
	38 ± 3	29 ± 4	47 ± 3	41 ± 4	59 ± 4	57 ± 5
	41 ± 2	35 ± 3	64 ± 3	58 ± 4	76 ± 6	76 ± 4
3	60 ± 3	48 ± 5	93 ± 3	62 ± 6	96 ± 4	92 ± 6
	65 ± 5	45 ± 3	83 ± 7	51 ± 4	94 ± 6	66 ± 4
	42 ± 4	51 ± 2	53 ± 5	63 ± 3	75 ± 8	89 ± 3
	38 ± 3	49 ± 2	55 ± 2	67 ± 4	75 ± 3	88 ± 5
	42 ± 2	37 ± 3	61 ± 3	50 ± 4	75 ± 2	81 ± 3
4	35 ± 3	27 ± 3	50 ± 4	63 ± 5	74 ± 2	73 ± 4
	65 ± 6	48 ± 3	89 ± 6	71 ± 4	101 ± 4	76 ± 7
	36 ± 4	41 ± 3	40 ± 4	65 ± 3	56 ± 4	73 ± 2
	25 ± 4	28 ± 3	33 ± 2	36 ± 3	44 ± 3	66 ± 6
	46 ± 3	41 ± 3	57 ± 2	46 ± 5	80 ± 3	65 ± 3
5	44 ± 3	58 ± 4	65 ± 2	90 ± 6	79 ± 2	77 ± 1
	84 ± 5	102 ± 9	89 ± 7	100 ± 8	99 ± 7	102 ± 3
	44 ± 5	30 ± 3	82 ± 6	33 ± 3	93 ± 5	67 ± 3
	46 ± 3	41 ± 4	51 ± 2	55 ± 4	80 ± 3	96 ± 5
	66 ± 2	68 ± 3	87 ± 2	82 ± 2	100 ± 3	95 ± 3
6	65 ± 2	55 ± 3	80 ± 2	70 ± 4	91 ± 4	86 ± 5
	89 ± 8	48 ± 3	100 ± 7	54 ± 5	100 ± 6	74 ± 5
	49 ± 4	79 ± 4	80 ± 6	102 ± 6	100 ± 5	101 ± 6
	51 ± 2	63 ± 3	69 ± 2	72 ± 3	84 ± 2	86 ± 4
	57 ± 2	47 ± 4	77 ± 2	76 ± 3	89 ± 3	94 ± 5
7	83 ± 2	68 ± 2	87 ± 1	80 ± 1	94 ± 2	96 ± 1
	78 ± 4	57 ± 3	91 ± 4	72 ± 2	99 ± 5	97 ± 2
	38 ± 3	41 ± 3	56 ± 4	64 ± 2	89 ± 4	78 ± 3
	76 ± 3	78 ± 4	86 ± 3	83 ± 4	89 ± 3	85 ± 5
	68 ± 3	70 ± 3	75 ± 3	86 ± 4	98 ± 5	94 ± 5
8	54 ± 4	56 ± 6	73 ± 6	76 ± 6	90 ± 7	92 ± 6
	101 ± 10	50 ± 6	93 ± 6	66 ± 6	97 ± 7	80 ± 7
	40 ± 2	41 ± 4	53 ± 2	56 ± 2	56 ± 3	70 ± 5
	56 ± 2	57 ± 5	69 ± 4	69 ± 2	78 ± 2	82 ± 4
	57 ± 2	58 ± 4	72 ± 2	74 ± 5	83 ± 4	86 ± 5
9	83 ± 6	77 ± 5	97 ± 7	88 ± 7	100 ± 8	96 ± 6
	98 ± 6	66 ± 5	92 ± 8	77 ± 6	98 ± 8	87 ± 8
	60 ± 3	62 ± 3	73 ± 4	75 ± 5	83 ± 4	85 ± 6
	69 ± 5	72 ± 4	83 ± 3	85 ± 4	96 ± 5	97 ± 6
	72 ± 7	74 ± 5	83 ± 5	88 ± 6	95 ± 9	97 ± 5
10	73 ± 2	90 ± 3	87 ± 2	95 ± 1	88 ± 3	96 ± 9
	101 ± 8	98 ± 7	99 ± 4	102 ± 7	100 ± 3	93 ± 10
	36 ± 2	41 ± 2	42 ± 3	72 ± 2	64 ± 4	76 ± 3
	42 ± 3	64 ± 4	55 ± 4	78 ± 5	71 ± 3	88 ± 5
	61 ± 2	47 ± 2	74 ± 4	68 ± 4	80 ± 2	85 ± 3
11	90 ± 3	80 ± 5	91 ± 5	83 ± 5	95 ± 4	97 ± 8
	101 ± 8	98 ± 7	99 ± 5	101 ± 7	101 ± 2	99 ± 10
	49 ± 4	46 ± 3	65 ± 5	67 ± 3	87 ± 5	79 ± 3
	57 ± 2	66 ± 2	75 ± 2	78 ± 3	86 ± 4	91 ± 4
	69 ± 3	72 ± 3	87 ± 3	80 ± 3	94 ± 4	91 ± 3
12	93 ± 3	95 ± 4	94 ± 3	101 ± 3	95 ± 3	100 ± 4
	102 ± 11	97 ± 2	102 ± 10	92 ± 4	97 ± 7	100 ± 2
	60 ± 5	65 ± 6	78 ± 6	69 ± 3	93 ± 4	88 ± 3
	59 ± 3	69 ± 6	82 ± 2	86 ± 4	89 ± 3	90 ± 2
	80 ± 4	76 ± 3	92 ± 2	89 ± 5	99 ± 3	90 ± 3
13	39 ± 2	45 ± 3	59 ± 3	80 ± 3	89 ± 6	88 ± 6
	76 ± 6	38 ± 2	90 ± 7	56 ± 3	103 ± 2	62 ± 3
	37 ± 5	41 ± 3	53 ± 6	37 ± 3	65 ± 4	67 ± 3
	51 ± 3	47 ± 3	54 ± 3	62 ± 3	60 ± 3	76 ± 4
	45 ± 2	38 ± 4	59 ± 2	64 ± 3	66 ± 2	79 ± 3
14	40 ± 2	45 ± 4	61 ± 2	82 ± 6	88 ± 3	90 ± 8
	74 ± 5	49 ± 2	91 ± 6	52 ± 3	92 ± 3	66 ± 5
	42 ± 4	36 ± 3	50 ± 4	50 ± 3	55 ± 5	74 ± 3
	40 ± 3	50 ± 4	63 ± 4	60 ± 5	80 ± 2	76 ± 5
	37 ± 2	42 ± 3	57 ± 2	60 ± 3	75 ± 2	79 ± 3

Results are given as means ± SE from 3 replicates.
Test plants were cockscomb, lettuce, cress, rice, and perennial ryegrass; the results are presented in the order of cockscomb, lettuce, cress, rice, and perennial ryegrass for each compound.

galls of *H. rhombea* are a conjugated diyne and a terminal olefinic group connecting to the aliphatic chain and that less oxidized acetylenes enhanced the activity except for stronger inhibition of the diepoxide.

Experimental

General procedures

Optical rotations were measured with a JASCO DIP-370 polarimeter. IR spectra were recorded on a JASCO FT/IR-300 spectrometer. ^{1}H and ^{13}C NMR spectra were measured and recorded in $CDCl_3$ or CD_3OD on a Bruker Avance 500 or 600 spectrometer. Chemical shift values (δ) are reported in parts per million (ppm) relative to the NMR solvents $CDCl_3$ (δ_H 7.26, δ_C 77.0) or CD_3OD (δ_H 3.35, δ_C 49.8). FAB mass spectra were recorded on a JMS-SX102/GCG spectrometer with xenon atoms, and *m*-nitrobenzylalcohol (NBA) was used as a matrix. ESI mass spectra were recorded on a Waters Platform LC mass spectrometer. GC mass spectra was recorded on a JEOL MS route MS-600 instrument. Helium was used as the carrier gas. The injection was performed at an oven temperature of 40 °C. After 1 min, the temperature was increased with a rate of 15 °C/min up to 250 °C and held for 5 min.

Plant material

Galls of *Hedera rhombea* induced by infection of *Asphondylia* sp. were collected at Tsuchiura city, Japan. A voucher specimen has been deposited at the Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan.

Extraction and isolation

Galls of *H. rhombea* (110 g) were homogenized by a blender, extracted with MeOH (420 mL) and concentrated *in vacuo*. The MeOH extracts (9.37 g) were partitioned between EtOAc (500 mL × 3) and H_2O (500 mL) and the H_2O-layer was further partitioned with BuOH (500 mL × 3). EtOAc-soluble portion (1.24 g) was chromatographed on silica gel using hexane/EtOAc (19:1 → 0:1) as the eluting solvents and separated into 12 fractions (EA-1 ~ EA-12). Fraction EA-5 (159 mg) was subjected to an ODS column (Waters, Sep-Pak Vac 35cc C_{18}-10 g) eluted with MeOH/H_2O (9:1 → 0:1) to give 9 fractions (EA-5-1 ~ EA-5-9). Fraction EA-5-3 (53.4 mg) was then subjected to reversed-phase HPLC (TSK-GEL ODS-80Ts, 7.8 mm × 30.0 cm, flow rate 2.0 mL/min, MeOH/H_2O, 85:15 v/v) to give **1** (10.2 mg, t_R 18 min). Fraction EA-5-4 (13.9 mg) was subjected to reversed-phase HPLC (TSK-GEL ODS-80Ts, 7.8 mm × 30.0 cm, flow rate 2.0 mL/min, MeOH/H_2O, 87:13 v/v) to give **2** (5.4 mg, t_R 21 min) and **3** (0.5 mg, t_R 23 min). Fraction EA-6 (75.0 mg) was subjected to reversed-phase HPLC (TSK-GEL ODS-80Ts, 7.8 mm × 30.0 cm, flow rate 2.0 mL/min, MeOH/H_2O, 85:15 v/v) to give **4** (6.3 mg, t_R 9 min) and **5** (55.9 mg, t_R 11 min). Fraction EA-7 (30.5 mg) was subjected to reversed-phase HPLC (TSK-GEL ODS-80Ts, 7.8 mm × 30.0 cm, flow rate 2.0 mL/min, MeOH/H_2O, 75:25 v/v), followed by preparative TLC (*n*-hexane/EtOAc, 2:1 v/v) to give **6** (1.0 mg). Fraction EA-8 (16.6 mg) was subjected to an ODS column (Waters, Sep-Pak Vac 12cc C_{18}-2 g) eluted with MeOH/H_2O (7:1 → 0:1 v/v) to give **7** (0.7 mg, t_R 19 min). BuOH-soluble portions (2.34 g) were subjected to an ODS column (Nacalai Tesque, Cosmosil 75 C_{18}-*PREP*, 22 mm × 30.0 cm) eluted with MeOH/H_2O (3:2 → 1:0 v/v) to give 19 fractions (BU-1 ~ BU-19). Fraction BU-9 (50.5 mg) was subjected to reversed-phase HPLC (TSK-GEL ODS-80Ts, 7.8 mm × 30.0 cm, flow rate 2.0 mL/min, MeOH/H_2O, 85:15 v/v) followed by separation by reversed-phase HPLC [TSK-GEL ODS-80Ts, 4.6 mm × 25.0 cm, flow rate 1.0 mL/min, gradient mobile phase (CH_3CN/H_2O, 50:50 → 100:0 v/v) in 50 min] to give **8** (0.7 mg, t_R 24 min) and **9** (0.8 mg, t_R 25 min). Fraction BU-10 (22.4 mg) was subjected to reversed-phase HPLC (Develosil ODS HG-5, 8.0 mm × 20.0 cm, flow rate 2.5 mL/min, CH_3CN/H_2O, 65:35 v/v), followed by preparative TLC (*n*-hexane/EtOAc, 3:1 v/v) to give **10** (0.7 mg) and **11** (1.2 mg). Fraction BU-11 (12.6 mg) was subjected to reversed-phase HPLC (TSK-GEL ODS-80Ts, 7.8 mm × 30.0 cm, flow rate 2.0 mL/min, MeOH/H_2O, 85:15 v/v) to give **12** (2.4 mg, t_R 18 min). Information in detail on analytical data of compounds **1**–**14** and work-up procedure of derivatization of **13** and **14** are obtainable from the author of correspondence.

Bioassays

Ten seeds of cockscomb (*Celosia argentea* L.), lettuce (*Lactuca sativa* L.), cress (*Lepidium sativum* L.), rice (*Oryza sativa* L.), and perennial ryegrass (*Lolium perenne* L.) were placed on a filter paper (No. 1, Toyo) moistened with 500 μL of test solution (10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} M for each acetylene) in a 2.8-cm (cockscomb, lettuce, cress, and perennial ryegrass) or 3.3-cm (rice) Petri dish and kept for 5, 3, 2, 3, and 4 d, respectively, at 25 °C in the dark, after which the lengths of their roots and shoots were measured and the percentage elongation of the roots was determined by reference to the elongation of control roots and shoots. 0.01% (v/v) aqueous solution of Triton X-100 was used for dilution of samples.

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