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Growth inhibitory indole acetic acid polyacetylenic ester from Japanese ivy (*Hedera rhombea* Bean)

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Abstract

Polyacetylenes **1** and **2** were isolated from extracts of Japanese ivy (*Hedera rhombea* Bean) flower buds, with their chemical structures established on the basis of extensive 1D and 2D NMR and MS analyses. The absolute configurations of compounds **1** and **2** were determined by both chemical means, and by using the modified Mosher's method. Compound **1** is the first polyacetylene having an ester linkage between falcarindiol (**3**) and indole-3-acetic acid (IAA) moieties and **2** also had an unique substructure containing a conjugated diene adjacent to a hydroxy group. Polyacetylenes **1**, **2**, and **3** were also subjected to assessment of growth inhibition against the shoot and root growth of the monocotyledon plants, rice and perennial ryegrass, as well as the dicotyledons, cockscomb, lettuce, cress, and fenugreek. The most bioactive compound appeared to be compound **1**, while **2** showed no activity. Compound **1** selectively showed growth inhibitory activity against dicotyledons.
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Keywords: *Hedera rhombea*; Araliaceae; Gall; Plant-growth inhibitor; Polyacetylene

1. Introduction

Hedera rhombea Bean (Kizuta in Japanese) is an evergreen vine in the family Araliaceae, which is widely distributed in Japan, Korea, and China; several fatty acids (Kuromo and Sakai, 1954) and saponins (Kizu et al., 1985) have been reported as the constituents of this plant. However, little is known on the acetylenic constituents of this plant except for our previous reports (Yamazoe et al., 2006b), where screening for bioactive compounds in galls of *H. rhombea* was conducted, resulting in isolation of new falcarindiol-related polyacetylenes. In the course of the current investigation on the constituents of flower buds of *H. rhombea*, two new polyacetylenes **1** and **2** were isolated and characterized. Since falcarindiol was known to exhibit plant-growth inhibitiory acitivity (Satoh et al., 1996), the bioactivities of **1** and **2** on plant growth were also examined.

In this study, we report the isolation and structure elucidation of new polyacetylenes **1** and **2** and their biological activities on the growth of several plants including weeds.

2. Results and discussion

2.1. Isolation and structure elucidation of compounds 1 and 2

The acetone extract of flower buds of *H. rhombea* was suspended in MeOH and MeOH-soluble portion was removed. The remaining MeOH-insoluble portion was repeatedly subjected to silica gel column chromatography, followed by ODS HPLC, to give compound **1** (0.0005%). The MeOH extract of the flower buds was partitioned between EtOAc and water, and the EtOAc-soluble portion was separated by SiO_2 column chromatography followed by ODS HPLC to afford compound **2** (0.0026%) (Fig. 1).

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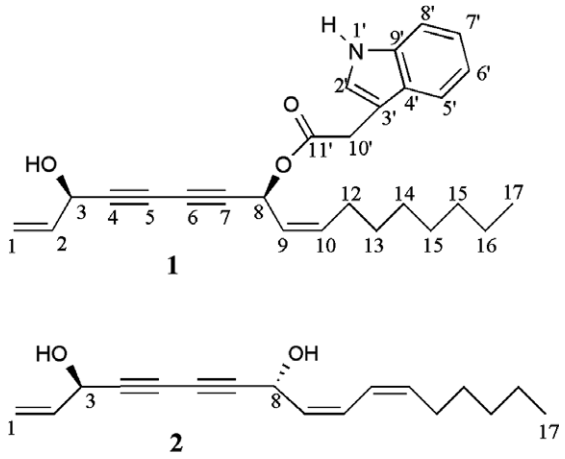

Fig. 1. Structures of polyacetylenes **1** and **2**.

Although compounds **1** and **2** were obtained as yellow oils, they gradually became brown (oxidised) on standing at room temperature. Compound **1** was isolated as an optically active oil, $[\alpha]_D^{26} + 43$ (*c* 0.4, $CHCl_3$), and was assigned as $C_{27}H_{31}NO_3$ by the HRFABMS [*m/z* 440.2209 $(M+Na)^+$, Δ+0.7 mmu]. Its broad IR absorption band at 3423 cm^{-1} was indicative of NH and/or OH groups, and those at 1701, 1654, 1637, 1542, and 1458 cm^{-1} were indicative of carbonyl, phenyl group and double bonds. The series of UV absorptions at 221, 271, and 281 nm, was in good agreement with published data for diacetylenes (Jung et al., 2002), whereas the sharp absorption at 289 nm suggested the presence of an indole ring. The ^{13}C NMR and HMQC experiments indicated the presence of 27 carbons, one methyl, eight methylenes, ten methines, and eight quaternary carbon signals, respectively (Table 1). In the ^1H NMR spectrum, resonances similar to those of falcarindiol (**3**) was observed, including a deshielded oxymethine at δ_H 6.19 (H-8), terminal olefinic protons at δ_H 5.93 (H-2), 5.47 (H-1a), and 5.26 (H-1b), olefinic protons at δ_H 5.65 (H-10) and δ_H 5.48 (H-9), an oxymethine at δ_H 4.93 (H-3), methylene protons at δ_H 2.11 (H-11), 1.33 (H-12), and 1.26 (H-13-16) and methyl protons at δ_H 0.88 (H-17), respectively; this interpretation was also supported by analysis of the ^{13}C NMR spectrum (Table 2) (Park and Kim, 1995). In addition to those resonances, signals for five aromatic protons at δ_H 8.07 (H-2′), 7.59 (H-5′), 7.36 (H-8′), 7.20 (H-7′), and 7.13 (H-6′), and a lower-field shifted methylene at δ_H 3.80 (H-10′) were observed, which were very similar to those of 3-indoleacetate. The ^1H and ^{13}C NMR spectroscopic resonances of **1** were almost identical to those of falcarindiol (**3**) and 3-indoleacetate, suggesting compound **1** to be a conjugate. The ^{13}C NMR signal of C-8 (δ_C 60.4) was shifted 1.9 ppm lower than that of **3** (δ_C 58.5) and in the ^1H NMR spectrum, the resonance (δ_H 6.19) of H-8 was shifted 0.99 ppm lower than that of **3** (δ_H 5.20), indicating that the 3-indoleacetoxy group connected to C-8 of falcarindiol (**3**). This linkage was confirmed by a clear cross-peak of the H-8 resonance with the C-11 signal in the HMBC (Fig. 2). Thus, **1** was concluded to be falcarindiol bearing a 3-indoleacetoxy group at C-8 position.

To confirm the chemical structure and to determine the absolute stereochemistries at C-3 and C-8 of **1**, falcarindiol (**3**), isolated from flower buds of *H. rhombea*, was deriva-

Table 1
^1H NMR (500 MHz) spectroscopic data of compounds **1** and **2** in $CDCl_3$ (ppm, *J* in Hz)

H	1	2
1a	5.47, *d* (16.8)	5.48, *d* (15.9)
1b	5.26, *d* (10.8)	5.27, *d* (10.1)
2	5.93, *ddd* (16.8, 10.8, 5.4)	5.94, *ddd* (15.9, 10.1, 5.7)
3	4.93 *brs*	4.94, *d* (5.7)
8	6.19 *d* (8.5)	5.37, *m*
9	5.48 *dd* (10.5, 8.5)	5.50 *dd* (9.9, 8.5)
10	5.65, *dt* (10.5, 7.5)	6.44 *dd* (11.1, 9.9)
11	2.11, *m*	6.24 *dd* (11.1, 10.9)
12	1.33, *t*-like (7.5)	5.66 *dt* (10.9, 8.6)
13	1.26, *m*	2.18, *q* (8.6)
14	1.26, *m*	1.39 *quit* (8.6)
15	1.26, *m*	1.29, *m*
16	1.26, *m*	1.29, *m*
17	0.87, *t* (7.3)	0.88, *t* (7.2)
1′	7.19, *brs*	
2′	8.07, *brs*	
5′	7.59, *d* (7.8)	
6′	7.13, *dd* (7.8, 7.3)	
7′	7.20, *dd* (7.5, 7.3)	
8′	7.36, *d* (7.5)	
10′	3.80, *s*	

Table 2
^{13}C NMR (125 MHz) spectroscopic data of compounds **1** and **2** in $CDCl_3$ (ppm)

Carbon	1	2
1	117.4	117.3
2	135.7	136.9
3	63.5	63.6
4	78.4	78.4
5	70.3	70.4
6	69.3	69.0
7	76.5	79.5
8	60.4	58.6
9	123.7	127.4
10	136.6	126.8
11	27.9	122.0
12	29.2	135.8
13	29.1	28.6
14	29.1	29.3
15	31.8	31.4
16	22.6	22.6
17	14.1	14.0
2′	123.7	
3′	107.9	
4′	127.2	
5′	118.9	
6′	119.7	
7′	122.2	
8′	111.1	
9′	136.0	
10′	31.1	
11′	170.4	

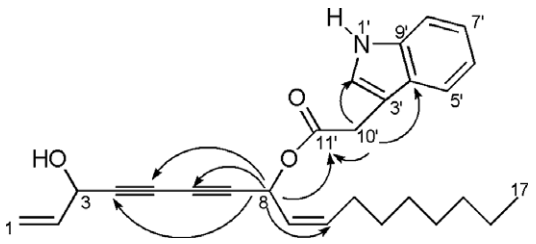

Fig. 2. Selected HMBC correlations for compound **1**.

tized. The absolute stereochemistry of **3** was next determined by the modified Mosher's method, aided by determination of its optical rotion value; the absolute configurations at C-3 and C-8 of **3** were thus concluded to be 3*R* and 8*S* (Ohtani et al., 1991; Ratnayake and Hemscheidt, 2002). The coupling of **3** with indole 3-acetic acid (IAA) was also performed by esterification by a base catalysis reacton between falcarindiol (**3**) and 3-indoleacetyl chloride prepared from IAA. As a result, the 8-monoester **1** was obtained together with the 3-monoester **4** and the diester **5** (Fig. 3). The synthetic product **1** had its optical rotation, ^{1}H NMR spectroscopic data and HPLC retention time matching those of natural product **1**. Hence the chemical structure of **1** was elucidated as (3*R*, 8*S*, *Z*)-3-hydroxyheptadeca-1,9-dien-4,6-diyn-8-yl 11-(1*H*-indol-3-yl)acetate.

Compound **2** was obtained as an oil, whose HRFABMS spectrum gave an ion peak at 281.3536 $(M+Na)^+$, suggesting a molecular formula of $C_{17}H_{22}O_2$. A strong UV maximum at 237 nm also indicated the presence of conjugated diene, whereas the IR spectrum of **2** had absorptions corresponding to hydroxyl groups (3440 cm^{-1}), double bonds (1635 cm^{-1}), and triple bonds (2368 and 2349 cm^{-1}), respectively. In the ^{1}H NMR spectrum, the terminal olefin protons at δ_H 5.94 (H-2), 5.48 (H-1a), and 5.27(H-1b), two conjugated olefinic signals at δ_H 6.44 (H-10), 5.50 (H-9) and at δ_H 6.24 (H-11), 5.66 (H-12), two oxymethines at δ_H 5.37 (H-8) and at δ_H 4.94 (H-3), besides methylene protons at δ_H 2.18 (H-13), 1.39 (H-14), and 1.29 (H-15, 16) and methyl protons at δ_H 0.88 (H-17) were observed (Table 1). The linkage of the two pairs of olefinic groups was confirmed by analysis of the ^{1}H–^{1}H COSY spectrum, in which the signal at δ_H 6.44 (H-10) was connected with another olefinic resonance at δ_H 6.24 (H-11). Conjugation of the two double bonds was supported by chemical shifts of H-10 (δ_H 6.44) and H-11 (δ_H 6.24), which were observed at lower-field compared with that of the typical isolated olefin signals, and both double bonds (H-9–H-10 and H-11–H-12) were assigned as a *Z* configuration on the basis of their coupling constants ($J_{9,10} = 9.9$ Hz and $J_{11,12} = 10.9$ Hz). Thus, analyses of the ^{1}H, ^{13}C NMR and ^{1}H–^{1}H COSY spectra permitted establishment of the sequence C-8–C-17 of the molecule, which was characterized by an alcohol function (C-8), a *cis* double bond (C-9–C-10), a conjugated *cis* double bond (C-11–C-12), four methylenes (C-13–C-16), and a methyl group (C-17), respectively. Further NMR spectroscopic analysis also permitted establishment of the remaining part of the molecule, which consisted of a terminal olefin (C-1–C-2) and a hydroxy group (C-3) moieties. In the ^{13}C NMR spectrum, four quaternary carbon signals at δ_C 79.5 (C-7), 78.4 (C-4), 70.4 (C-5), and 69.0 (C-6) were characteristically observed (Table 1). Fur-

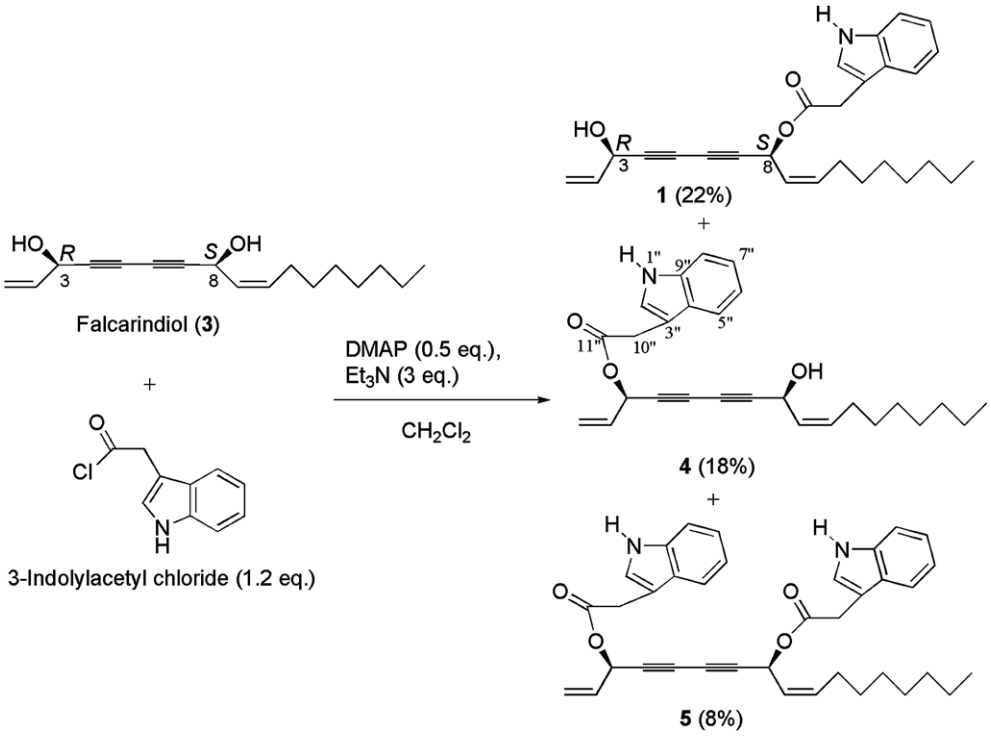

Fig. 3. Derivatization of **1** from **3**.

thermore, long-range correlations of H-2 to C-4, H-3 to C-4, H-3 to C-4 and C-5, H-8 to C-6 and C-7, and H-9 to C-7 suggested that C-1–C-3 and C-8–C-17 were linked by a conjugated diyne moiety (C-4–C-7). Therefore, the structure of **2** was elucidated as (9*Z*, 11*Z*)-heptadeca-1,9,11-triene-4,6-diyne-3,8-diol.

In order to also determine the absolute configurations at C-3 and C-8, the modified Mosher's method was applied on **2** (Ohtani et al., 1991). Compound **2** was treated with (*R*)- and (*S*)-α-methoxy-α-trifluoromethylphenylacetyl chloride (MTPA-Cl) in the presence of 4-dimethylaminopyridine (DMAP) and triethylamine to give the di-(*S*)- and di-(*R*)-MTPA esters (**2a** and **2b**), respectively. In the ^{1}H NMR spectrum of the di-(*S*)-MTPA ester **2a**, the proton signals assigned to H-1, H-2, H-9, H-10, H-11, and H-12 were observed at lower-field than those of the di-(*R*)-MTPA ester **2b**, indicating that C-3 and C-8 both had *R*-configurations (Fig. 4).

2.2. Growth inhibitory activity of compounds 1 and 2

Since it had been shown that falcarindiol (**3**) had potent plant-growth inhibitory activity (Satoh et al., 1996), the effect of the isolated polyacetylenes **1**, **2**, and **3** from flower buds of *H. rhombea* were studied for inhibition of shoot and root growth of the monocotyledonous plants, rice, perennial ryegrass, fenugreek and dicotyledonous ones, cockscomb, lettuce, and cress in each test solution of 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} M (Table 3). Compounds **1**–**3** generally showed inhibitory effects on both root and shoot growth of used plants in a dose-dependent manner except for rice growth. Compound **1** exhibited growth inhibitory activity selectively against dicotyledonous plants, whereas it showed no or less inhibition against monocotyledons. As for the dicotyledon plants, the most active compound appeared to be compound **1**, followed by compound **3**, and then **2**. This observation that the inhibitory activity against dicotyledonous plants of **1** was higher than that of falcarindiol (**3**) suggested that IAA at C-8 is important for selective plant-growth inhibitory activity. On the other hand, compound **2** showed no inhibitory activities ($>1\times10^{-4}$). This result was, therefore, in accord with the findings obtained in the preceding study (Yamazoe et al., 2006a), which was that the presence of conjugated double bonds at C-9, C-10 and C-11, C-12 reduced the plant-growth inhibitory activity.

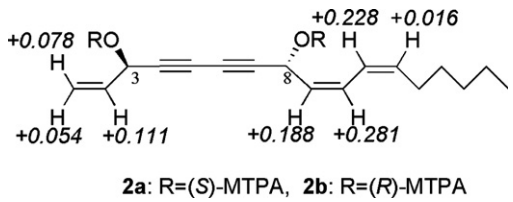

Fig. 4. Δδ values [Δδ (in ppm) = $\delta_S - \delta_R$] obtained for the (*S*)- and (*R*)-MTPA esters (**2a** and **2b**, respectively) of **2**.

Table 3
Inhibitory activity of polyacetylenes on the growth of roots and shoots of test plants

Test plants	Compounds	EC_{50} (M)	
		Root	Shoot
Dicotyledons			
Lettuce	**1**	3×10^{-5}	7×10^{-6}
	2	$>1\times10^{-4}$	$>1\times10^{-4}$
	3	$>1\times10^{-4}$	$>\times10^{-4}$
Cockscomb	**1**	4×10^{-6}	9×10^{-6}
	2	$>1\times10^{-4}$	$>1\times10^{-4}$
	3	5×10^{-5}	$>1\times10^{-4}$
Cress	**1**	8×10^{-7}	3×10^{-5}
	2	$>1\times10^{-4}$	$>1\times10^{-4}$
	3	8×10^{-5}	3×10^{-6}
Fenugreek	**1**	9×10^{-8}	2×10^{-7}
	2	$>1\times10^{-4}$	$>1\times10^{-4}$
	3	$>1\times10^{-4}$	4×10^{-4}
Monocotyledons			
Rice	**1**	$>1\times10^{-4}$	$>1\times10^{-4}$
	2	$>1\times10^{-4}$	$>1\times10^{-4}$
	3	1×10^{-5}	2×10^{-5}
Perennial ryegrass	**1**	$>1\times10^{-4}$	$>1\times10^{-4}$
	2	$>1\times10^{-4}$	$>1\times10^{-4}$
	3	$>1\times10^{-4}$	$>1\times10^{-4}$

3. Experimental section

3.1. General experimental procedures

Optical rotations were measured with a JASCO DIP-370 polarimeter. UV spectra were recorded on a HITACHI U-2000A spectrometer, whereas IR spectra were acquired using a JASCO FT/IR-300 spectrometer. The ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were measured and recorded in $CDCl_3$ on a Bruker Avance 500 spectrometer. Chemical shift values (δ) were reported in parts per million (ppm) relative to the NMR solvent $CDCl_3$ (δ_H 7.26, δ_C 77.0). FABMS were recorded in the positive-ion mode on JEOL IMS-SX102 mass spectrometer.

3.2. Plant material

Flower buds of *H. rhombea* were collected at Tsuchiura city, Japan. A voucher specimen (UT-HR050526) has been deposited at the Graduate School of Life and Environmental Sciences, University of Tsukuba, Japan.

3.3. Extraction and isolation

Flower buds (140 g and 110 g) of *H. rhombea* were individually homogenized using a homogenizer (Nissei AM-10, Nihonseiki Kaisya Ltd., Japan), then successively extracted with acetone (500 mL) and MeOH (550 mL), and concentrated *in vacuo* to obtain acetone (3.99 g) and MeOH (10.5 g) extracts, respectively. To the acetone extract

(3.99 g) was added by MeOH (100 mL) to afford the MeOH-soluble and the MeOH-insoluble portions. The MeOH-insoluble portion (1.56 g) was subjected to silica gel CC (2.0 × 20 cm), eluted with a $CHCl_3$–EtOAc solvent system of increasing polarity ($CHCl_3$–EtOAc, 9:1 → 1:1) to afford 10 fractions (fr. 1 ~ fr. 10). Fr. 7 (60.5 mg, $CHCl_3$–EtOAc, 6:1) was further applied to a silica gel column (1.0 × 13 cm) eluted with *n*-hexane–EtOAc (9:1 → 1:2) to afford nine fractions. The fraction (4.3 mg) eluted with *n*-hexane–EtOAc (6:1) was separated by reversed-phase HPLC [Atlantis dC_{18} 5 μm, 4.6 mm× 25.0 cm, Waters, flow rate 1.0 mL/min, CH_3CN/H_2O, 85:15 v/v, detection UV (195, 214, 254, 280, and 300 nm)] to give **1** (0.7 mg, t_R 14.0 min). Fr. 8 (306 mg) was subjected to reversed-phase HPLC (TSK-GEL ODS-80Ts, 7.8 mm × 30.0 cm, Tosoh Co. Ltd., flow rate 2.0 mL/min, $MeOH/H_2O$, 85:15 v/v) to give **3** (78.7 mg, t_R 11.0 min).

The initial MeOH extract (10.5 g) was then partitioned between EtOAc (500 mL × 3) and H_2O (500 mL). The EtOAc-soluble portion (2.3 g) was subjected to silica gel CC (2.2 × 30 cm) eluted with solvents of increasing polarity (*n*-hexane–EtOAc, 19:1 → 0:1) to afford 12 fractions. The fraction (29.8 mg) eluted with *n*-hexane–EtOAc (9:1) was further separated by reversed-phase HPLC (TSK-GEL ODS-80Ts, 7.8 mm × 30.0 cm, flow rate 2.0 mL/min, $MeOH/H_2O$, 85:15) to afford **2** (3.7 mg, t_R 10 min).

3.4. (*Z*)-3-Hydroxyheptadeca-1,9-dien-4,6-diyn-8-yl 11-(1*H*-indol-3-yl)acetate (*1*)

Colorless oil. $[\alpha]_D^{26}$ + 43 (*c* 0.4, $CHCl_3$). UV (Et_2O) λ_{max} (log ε) nm: 221 (4.4), 271 (4.0), 281 (3.9), 289 (3.9). IR (film) ν_{max} cm^{-1}: 3423, 2925, 2372, 2345, 1701, 1654, 1637, 1542, 1458. For ^1H and ^{13}C NMR spectroscopic data see Tables 1 and 2. ^1H–^1H COSY connectivities ($CDCl_3$, H/H) 1/2, 1/3, 3/8, 8/9, 8/10, 9/10, 10/11, 11/12, 16/17, 1′/2′, 5′/6′, 5′/7′, 6′/7′, 7′/8′. HMBC correlations ($CDCl_3$, H/C) 1/2, 1/3, 2/3, 3/1, 3/2, 8/4, 8/5, 8/6, 8/9, 8/10, 8/11′, 9/10, 9/11, 10/8, 10/11, 10/12, 11/9, 11/10, 11/12, 12/10, 12/13, 13/11, 13/12, 13/15, 14/12, 14/15, 14/16, 15/13, 15/16, 15/17, 16/14, 16/15, 16/17, 17/15, 17/16, 1′/3′, 1′/4′, 5′/3′, 5′/7′, 5′/9′, 6′/4′, 6′/8′, 7′/5′, 7′/8′, 7′/9′, 8′/4′, 8′/6′, 8′/7′, 10′/2′, 10′/3′, 10′/4′, 10′/11′. FABMS in NBA *m/z* 440 $[M+Na]^+$. HRFABMS in NBA *m/z* 440.2209 $[M+Na]^+$ (calcd for $C_{27}H_{31}NO_3Na$, 440.2202).

3.5. (9*Z*, 11*Z*)-Heptadeca-1,9,11-triene-4,6-diyne-3,8-diol (*2*)

Colorless oil. $[\alpha]_D^{23}$ + 96 (*c* 0.5, MeOH). UV (Et_2O) λ_{max} nm (log ε) 204 (4.2), 237 (4.1), 283 (3.5). IR (KBr) ν_{max} cm^{-1}: 3440, 2923, 2368, 2349, 1635. For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2. ^1H–^1H COSY connectivities ($CDCl_3$, H/H) 1/2, 1/3, 3/8, 8/9, 8/10, 9/10, 10/11, 11/12, 12/13, 13/14, 14/15, 16/17. HMBC correlations ($CDCl_3$, H/C) 1/2, 1/3, 2/3, 2/4, 3/1, 3/2, 3/4, 3/5, 8/6, 8/7, 8/9, 8/10, 9/7, 9/8, 9/11, 10/8, 10/11, 10/12, 11/9, 11/13, 12/10, 12/13, 12/14, 13/11, 13/12, 13/14, 13/15, 14/12, 14/13, 14/15, 14/16, 15/13, 15/14, 15/16, 15/17, 16/14, 16/15, 16/17, 17/15, 17/16, FABMS *m/z* 281 $[M+Na]^+$. HRFABMS *m/z* 281.3636 $[M+Na]^+$ (calcd for $C_{17}H_{22}O_2Na$, 281.3671).

3.6. Derivatization of 1 from falcarindiol (*3*)

Preparation of 3-indolylacetyl chloride followed the procedure of Stanley et al. (Stanley et al., 1987). 3-Indolylacetyl chloride (55.3 mg, 1.2 eq.), DMAP (14.6 mg, 0.5 eq.), and triethylamine (100 μL, 3 eq.) were added to a solution of falcarindiol (**3**, 62.1 mg, 0.24 mmol) in anhydrous CH_2Cl_2 (6.2 mL). After stirring at room temperature for 5 h, the reaction solution was successively washed with H_2O (1.5 mL), 5% Na_2CO_3 aq. (1.5 mL), and saturated NaCl aq. (1.5 mL). The solution was dried (anhydr. $MgSO_4$) and filtered, and the filtrate was concentrated under reduced pressure. Purification of the resulting residue by silica gel CC ($CHCl_3$ → $CHCl_3$–EtOAc, 7:1), followed by preparative silica gel thin layer chromatography ($CHCl_3$–EtOAc, 9:1) gave compounds **1** (13.7 mg, 22.2%), **4** (11.4 mg, 18.4 %), and **5** (4.9 mg, 7.9 %), respectively.

Compound **4**: colorless oil. $[\alpha]_D^{22}$ + 151 (*c* 0.8, $CHCl_3$). UV (Et_2O) λ_{max} nm (log ε) 221 (4.4), 270 (4.0), 279 (4.0), 289 (3.9). IR (film) ν_{max} cm^{-1} 3421, 2924, 2367, 2344, 1734, 1701, 1685, 1637, 1560, 1457. ^1H NMR ($CDCl_3$): δ 8.00 (1H, *brs*, H-2″), 7.61 (1H, *d*, *J* = 7.3 Hz, H-5″), 7.37 (1H, *d*, *J* = 8.1 Hz, H-8″), 7.19 (1H, *dd*, *J* = 8.5, 8.1 Hz, H-7″), 7.91 (1H, *brs*, H-1″), 7.14 (1H, *dd*, *J* = 8.5, 7.3 Hz, H-6″), 5.96 (1H, *d*, *J* = 5.7 Hz, H-3), 5.85 (1H, d*dd*, *J* = 16.8, 10.1, 5.7 Hz, H-2), 5.62 (1H, *dt*, *J* = 10.5, 7.2 Hz, H-10), 5.51 (1H, *dd*, *J* = 10.5, 8.5 Hz, H-9), 5.50 (1H, *d*, *J* = 16.8 Hz, H-1b), 5.31 (1H, *d*, *J* = 10.1 Hz, H-1a), 5.20 (1H, *d*, *J* = 8.5 Hz, H-8), 3.82 (1H, s, H-10″), 2.11 (2H, *m*, H-11), 1.38 (2H, t-like, *J* = 7.2 Hz, H-12), 1.27 (8H, *m*, H-13-16), 0.88 (3H, *t*, *J* = 7.5 Hz, H-17). ^{13}C NMR ($CDCl_3$): δ 170.4 (C-11″), 136.0 (C-9″), 134.7 (C-10), 131.8 (C-2), 127.5 (C-9), 127.1 (C-4″), 123.1 (C-2″), 122.3 (C-7″), 119.7 × 2 (C-1, C-6″), 118.8 (C-5″), 111.1 (C-8″), 107.8 (C-3″), 79.9 (C-7), 74.8 (C-4), 70.9 (C-5), 68.6 (C-6), 64.7 (C-3), 58.5 (C-8), 31.8 (C-15), 31.1 (C-10″), 29.2 (C-12), 29.1 × 2 (C-13, C-14), 27.8 (C-11), 22.6 (C-16), 14.1 (C-17). FABMS in NBA *m/z* 440 $[M+Na]^+$. HRFABMS in NBA *m/z* 440.2207 $[M+Na]^+$ (calcd for $C_{27}H_{31}NO_3Na$, 440.2202).

Compound **5**: colorless oil. $[\alpha]_D^{21}$ + 72 (*c* 2.0, $CHCl_3$). UV (Et_2O) λ_{max} nm (log ε) 221 (4.6), 272 (4.3), 279 (4.3), 289 (4.2). IR (film) ν_{max} cm^{-1} 3411, 2931, 2367, 2344, 1735, 1637, 1458. ^1H NMR ($CDCl_3$): δ 8.07 (2H, *brs*, H-2′, 2″), 7.60 (1H, *d*, *J* = 7.6 Hz, H-5″), 7.59 (1H, *d*, *J* = 7.6 Hz, H-5′), 7.36 (1H, *d*, *J* = 8.2 Hz, H-8″), 7.35 (1H, *d*, *J* = 8.1 Hz, H-8′), 7.19 (2H, *m*, H-7′, H-7″), 7.18 (2H, *brs*, H-1′, 1″), 7.14 (1H, *dd*, *J* = 7.7, 7.6 Hz, H-6″), 7.13 (1H, *dd*, *J* = 7.7, 7.6 Hz, H-6′), 6.18 (1H, *d*, *J* = 8.6 Hz, H-8), 5.95 (1H, *d*, *J* = 5.7 Hz, H-3), 5.85 (1H, d*dd*, *J* = 16.5, 10.1, 5.7 Hz, H-2), 5.66 (1H, *dt*, *J* = 10.1,

7.5 Hz, H-10), 5.47 (1H, *dd*, $J = 10.1$, 8.6 Hz, H-9), 5.51 (1H, *d*, $J = 16.5$ Hz, H-1b), 5.32 (1H, *d*, $J = 10.1$ Hz, H-1a), 3.83 (1H, *s*, H-10″), 3.81 (1H, *s*, 10′), 2.11 (2H, *m*, H-11), 1.34 (2H, t-like, $J = 7.2$ Hz, H-12), 1.27 (8H, *m*, H-13-16), 0.87 (3H, *t*, $J = 6.9$ Hz, H-17). ^{13}C NMR ($CDCl_3$): δ 170.4 × 2 (C-11′, 11″), 136.0 × 2 (C-9′, 9″), 136.7 (C-10), 131.7 (C-2), 123.5 (C-9), 127.1 × 2 (C-4′, 4″), 123.1 (C-2′), 123.0 (C-2″), 122.3 (C-7″), 122.2 (C-7′), 119.8 (C-1), 119.7 × 2 (C-6′, 6″), 118.8 × 2 (C-5′, 5″), 111.2 (C-8′), 111.1 (C-8″), 107.9 (C-3′), 107.9 (C-3″), 80.0 (C-7), 74.8 (C-4), 70.9 (C-5), 68.6 (C-6), 64.7 (C-3), 58.5 (C-8), 31.8 (C-15), 31.1 × 2 (C-10′, 10″), 29.2 (C-12), 29.1 × 2 (C-13, C-14), 27.7 (C-11), 22.6 (C-16), 14.1 (C-17). FABMS in NBA *m/z* 597 $[M+Na]^+$. HRFABMS in NBA *m/z* 597.2712 $[M+Na]^+$ (calcd for $C_{37}H_{38}N_2O_4Na$, 597.2720).

3.7. Preparation of (*R*)-MTPA ester (**2a**) from **2**

(*S*)-MTPA chloride (3.2 μL, 4 eq.), DMAP (0.26 mg), and Et_3N (1.8 μL, 3 eq.) were added to a solution of compound **2** (1.1 mg) in anhydrous CH_2Cl_2 (150 μL). After stirring at room temperature for 3.5 h, *N*, *N*-dimethyl-1,3-propanediamine (0.6 μL) was added to the reaction mixture and stirred at room temperature for further 10 min. The resulting mixture was purified by silica gel PTLC (*n*-hexane–EtOAc, 6:1) to give the di-(*R*)-MTPA ester (**2a**, 2.2 mg, 74%) as colorless oil. ^{1}H NMR ($CDCl_3$): δ 7.51 (4H, *m*, MTPA), 7.43 (6H, *m*, MTPA), 6.19 (1H, *d*, $J = 6.6$ Hz, H-8), 6.01 (1H, *d*, $J = 6.6$ Hz, H-3), 5.93 (1H, *dd*, $J = 11.1$, 10.5 Hz, H-10), 5.83 (1H, *dd*, $J = 11.1$, 10.3 Hz, H-11), 5.75 (1H, *dd*, $J = 16.9$, 10.2, 6.6 Hz, H-2), 5.70 (1H, *dt*, $J = 10.3$, 7.6 Hz, H-12), 5.53 (1H, *dd*, $J = 10.5$, 6.6 Hz, H-9), 5.41 (1H, *d*, $J = 16.9$ Hz, H-1), 5.24 (1H, *d*, $J = 10.1$ Hz, H-1), 3.67 (3H, s, MTPA-OMe), 3.47 (3H, *s*, MTPA-OMe), 2.19 (2H, q, $J = 7.6$ Hz, H-13), 1.37 (2H, quit, $J = 7.6$ Hz, H-14), 1.27 (4H, *m*, H-15, 16), 0.87 (3H, *t*, $J = 7.0$ Hz, H-17).

3.8. Preparation of (*S*)-MTPA ester (**2b**) of **2**

(*R*)-MTPA chroride (4.0 μL, 4 eq.), DMAP (0.33 mg), and triethylamine (2.3 μL, 3 eq.) were added to a solution of compound **2** (1.4 mg) in anhydrous CH_2Cl_2 (250 μL). Workup as described above gave the di-(*S*)-MTPA ester (**2b**, 2.1 mg, 56 %) as colorless oil. ^{1}H NMR ($CDCl_3$): δ 7.52 (4H, *m*, MTPA), 7.43 (6H, *m*, MTPA), 6.51 (1H, *d*, $J = 8.9$ Hz, H-8), 6.21 (1H, *dd*, $J = 11.1$, 10.2 Hz, H-10), 6.16 (1H, *d*, $J = 5.7$ Hz, H-3), 6.06 (1H, *dd*, $J = 11.1$, 9.9 Hz, H-11), 5.86 (1H, *ddd*, $J = 16.8$, 10.2, 5.7 Hz, H-2), 5.72 (1H, *dt*, $J = 9.9$, 7.6 Hz, H-12), 5.72 (1H, *dd*, $J = 10.1$, 8.9 Hz, H-9), 5.48 (1H, *d*, $J = 16.8$ Hz, H-1), 5.30 (1H, *d*, $J = 10.2$ Hz, H-1), 3.56 (3H, s, MTPA-OMe), 3.52 (3H, *s*, MTPA-OMe), 2.20 (2H, q, $J = 7.6$ Hz, H-13), 1.38 (2H, quit, $J = 7.6$ Hz, H-14), 1.27 (4H, *m*, H-15, 16), 0.87 (3H, *t*, $J = 7.6$ Hz, H-17).

3.9. Assay for plant-growth inhibitory activity

Ten seeds of lettuce (*Lactuca sativa* L.), cockscomb (*Celosia argentea* L.), cress (*Lepidium sativum* L.), rice (*Oryza sativa* L.), perennial ryegrass (*Lolium perenne* L.), and fenugreek (*Trigonella foenum-graecum* L.) were individually placed on filter papers (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, perennial ryegrass, and fenugreek) or 3.3-cm (rice) petri dish and kept for 5, 3, 2, 3, 4, and 2 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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