JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

Determination of the Absolute Configuration of a Monoglyceride Antibolting Compound and Isolation of Related Compounds from Radish Leaves (*Raphanus sativus*)

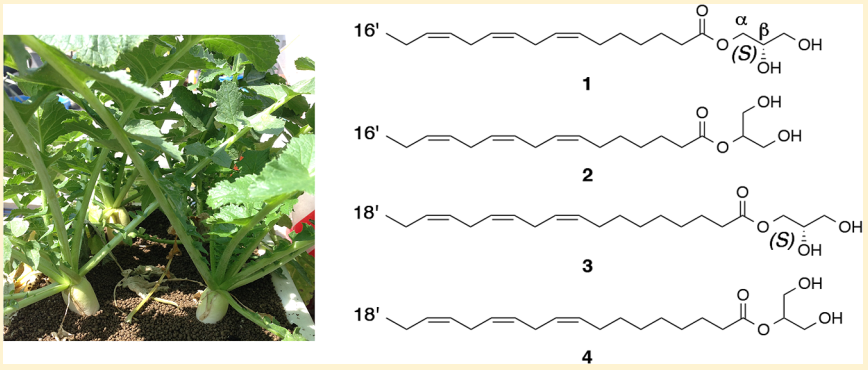
Tsuyoshi Ogihara,[†] Naruki Amano,[†] Yuki Mitsui,[‡] Kaien Fujino,[†] Hiroyuki Ohta,[§] Kosaku Takahashi,[†] and Hideyuki Matsuura*[,†]

[†]Research Faculty of Agriculture, Hokkaido University, Sapporo 060-8589, Japan

[‡]Tokyo University of Agriculture, 1-1-1, Sakuragaoka, Setagaya-ku, Tokyo 156-8502, Japan

[§]School of Life Science and Technology, Tokyo Institute of Technology, 4259-B65 Nagatsuta-cho, Midori-ku, Yokohama 226-8501, Japan

Supporting Information



ABSTRACT: A monoglyceride (**1**) has been reported to possess an antibolting effect in radish (*Raphanus sativus*), but its absolute configuration at the C-2 position was not determined earlier. In this work, the absolute configuration of **1** was determined to be (2*S*), and it was also accompanied by one new (**2**) and two known monoglycerides (**3** and **4**). The chemical structure of **2** was determined as *β*-(7′*Z*,10′*Z*,13′*Z*)-hexadecatrienoic acid monoglyceride (*β*-16:3 monoglyceride). Qualitative and quantitative analytical methods for compounds **1**−**4** were developed, using two deuterium-labeled compounds (**8** and **9**) as internal standards. The results revealed a broader range of distribution of **1**−**4** in several annual winter crops. It was also found that these isolated compounds have an inhibitory effect on the root elongation of *Arabidopsis thaliana* seedlings at concentrations of 25 and 50 *μ*M in the medium. However, the inhibitory effect of **1** was not dependent on coronatin-insensitive 1 (COI1) protein, which may suggest the involvement of an unidentified signaling system other than jasmonic acid signaling.

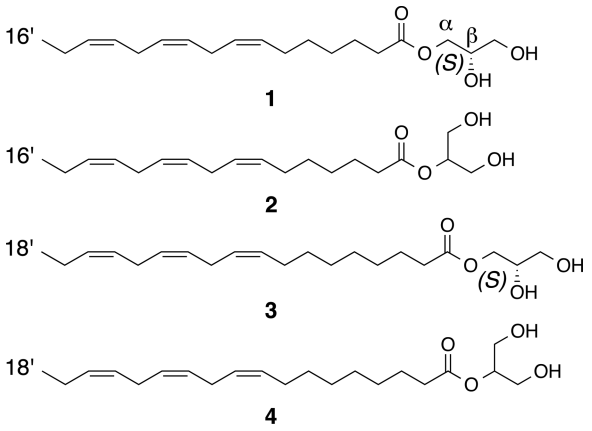
Plants respond to many environmental factors, such as temperature, the length of day, and availability or excess of water. In the autumn, radish (*Raphanus sativus* L.; Brassicaceae), spinach (*Spinacia oleracea*), beet (*Beta vulgaris*), and carrot (*Daucus carota*) grow leaves on a dwarf stem, at a level close to the ground. These structures are called rosettes. They accumulate their assimilated products in a thick root under short-day (SD) conditions and are categorized as cold-requiring long-day (LD) plants because a certain chilling period in the winter season is needed for initial stem growth. This effect occurs in response to the LD conditions of the next spring, in which the plant finally bears flowers on an elongated stem. This biological phenomenon, in which exposure to cold conditions is required for developing the initial stem from the storage organ, is known as vernalization,[1] and the elongation is known as bolting.[2,3] Thus, the life cycle of the cold-requiring LD plants can be summarized as follows: (i) the seeds germinate in the summer; (ii) the plants develop biomass in the rosette form to transport assimilated products into the storage organs and survive the winter by leaving the storage organs underground; and (iii) in the spring, the plants develop initial stems that are elongated to bear flowers at the top of the bolted stem. These flowers attract pollinators and then scatter the seeds in the summer. To perform the above-mentioned life cycle, plants must appropriately control growing and flowering times to ensure the formation of the next generation. Thus, biological regulation to inhibit bolting is a pivotal step for the plant to complete its life cycle, and extensive work has been conducted to uncover the mechanism of this process. In many cold-requiring LD plants, the exogenous application of gibberellic acid (GA) under short-day conditions induces bolting.

Received: August 12, 2016

ACS Publications © XXXX American Chemical Society and American Society of Pharmacognosy

DOI: 10.1021/acs.jnatprod.6b00746
J. Nat. Prod. XXXX, XXX, XXX−XXX

Chailakh[4] noted that the endogenous amount of GA in rosette plants under SD conditions is relatively low, and LD conditions should raise the level of endogenous GA to stimulate bolting. The exogenous application of inhibitor upon GA biosynthesis has led to adverse biological effects.[5,6] Yoshida et al.[7] predicted the existence of an anti-GA compound, or in other words, an antibolting compound (**1**), which is synthesized under SD conditions to inhibit stem elongation. They isolated α-(7′*Z*,10′*Z*,13′*Z*)-hexadecatrienoic acid monoglyceride (α-16:3 monoglyceride) (**1**) as the antibolting compound. However, they determined neither its absolute configuration nor its anti-GA activity. In this contribution, the absolute configuration of compound **1** is reported together with isolation of one new (**2**) and two known (**3** and **4**) monoglycerides from leaves of the radish (*R. sativus*). Also, qualitative and quantitative analytical methods were determined for compounds **1**−**4**, and their biological activities were evaluated against GA.



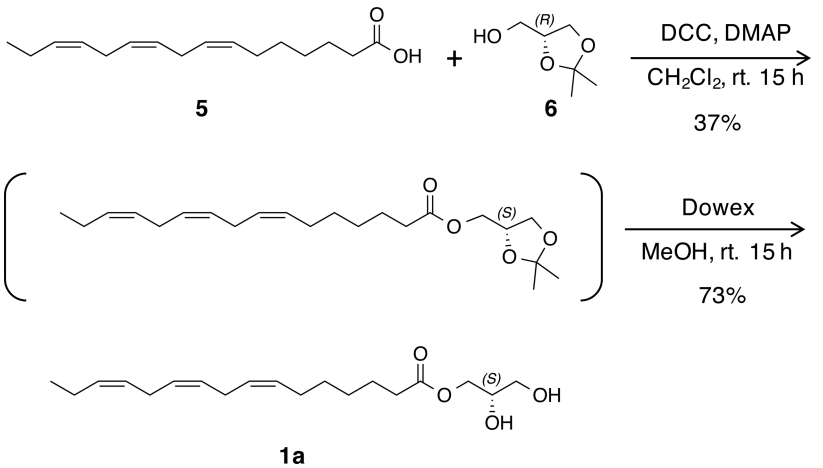
RESULTS AND DISCUSSION

First, the optically active antibolting compound **1** was obtained, using a synthetic compound as a standard for the purification steps. The fatty acid moiety of the standard compound, (7′*Z*,10′*Z*,13′*Z*)-hexadeca-7,10,13-trienoic acid (**5**), was synthesized according to a reported method,[8] but having 6-bromohexan-1-ol as a starting material. Then, compound **5** was coupled with an excess amount of propane-1,2,3-triol (**6**) to give synthetic **1** in the racemic mixture form. Subsequently, the naturally derived **1** (10.8 mg) was isolated from radish (*R. sativus*) leaves (Figure S1, Supporting Information). Additionally, three monoglycerides (**2**−**4**) were also isolated, which had retention times close to **1**, as shown by the corresponding features in the HPLC chromatogram (Figure S2, Supporting Information).

The isolated natural compound **1** showed an optical rotation value of $[\alpha]^{26}_{D}$ +2.4 (*c* 0.82, MeOH). (2*S*)- and (2*R*)-**1** were synthesized according to a previously reported method,[9] with the exception that **5** was used as the fatty acid moiety for conjugation with (*R*)-2,2-dimethyl-1,3-dioxolane-4-methanol (**6**, Scheme 1) and (*S*)-2,2-dimethyl-1,3-dioxolane-4-methanol (**7**, Scheme S1, Supporting Information). The optical rotation values of (2*S*)-α-(7′*Z*,10′*Z*,13′*Z*)-hexadecatrienoic acid monoglyceride (**1a**, Scheme 1) and (2*R*)-α-(7′*Z*,10′*Z*,13′*Z*)-hexadecatrienoic acid monoglyceride (**1b**, Scheme S1, Supporting Information) were measured as $[\alpha]^{26}_{D}$ +4.4 (*c* 0.75, MeOH) and −4.5 (*c* 0.75, MeOH), respectively, which revealed that the absolute configuration of the C-2 position of natural **1** might be in the *S* configuration. However, it was considered that natural **1** occurs in a racemic mixture to result into its lower optical rotation than that of the synthetic compound **1a**. In order to check the possibility that naturally derived **1** was obtained as a racemic mixture, the generation of (*S*)-MTPA esters composed of naturally derived **1**, **1a**, and **1b** (Figures S3−S5, Supporting Information) was conducted to evaluate the optical purity. It was observed that there were distinct differences in the resonances of ^{1}H NMR spectra between the (*S*)-MTPA esters of **1a** and **1b**, and the resonances of the ^{1}H NMR spectrum of the (*S*)-MTPA ester of **1** contained signals corresponding to the (*S*)-MTPA esters of **1a** and **1b** (Figure S6, Supporting Information). It was revealed that naturally derived **1** was a racemic mixture having an optical purity of 55%.

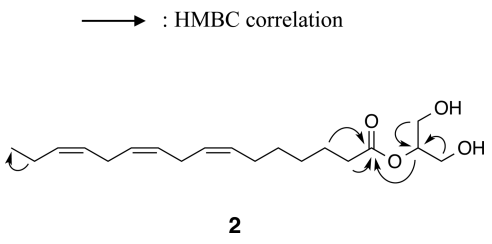
Compounds **2** (8.7 mg), **3** (21 mg), and **4** (6.9 mg) were isolated from an EtOH extract of radish leaves (2 kg). Compound **2** was isolated as a colorless oil, and its elemental formula, as determined by HRFDMS (Figure S7, Supporting Information), was found to be $C_{19}H_{32}O_4$, indicating four double-bond equivalents. The ^{1}H NMR spectrum (Figure S8, Supporting Information) showed a signal for one methyl group at δ_H 0.95 (3H, t, *J* = 7.6 Hz), six olefinic protons at δ_H 5.42−5.24 (6H, m), and five alkyl protons of glycerol at δ_H 3.81 (4H, d, *J* = 7.6 Hz) and 4.90 (1H, quintet, *J* = 4.6 Hz). One methyl

Scheme 1. Synthesis of (2*S*)-α-(7′*Z*,10′*Z*,13′*Z*)-Hexadecatrienoic Acid Monoglyceride (1a)



DOI: 10.1021/acs.jnatprod.6b00746
J. Nat. Prod. XXXX, XXX, XXX−XXX

carbon, three downfield oxygen-connected carbons including one methine and two equivalent methylene carbons, seven sp^2 carbons including one carbonyl group, and eight methylene carbons were observed in the ^{13}C NMR and DEPT spectra. Also observed were eight methylenes, six olefinic carbons, and one methyl carbon that showed an ω-3 fatty acid structure, which was supported by the data from the DEPT and HSQC experiments (Figures S9–S11, Supporting Information). The key correlations between carbons and protons ($J_{2,3}$) in the HMBC (Figure S12, Supporting Information) revealed an attachment between the carbonyl carbon and the primary carbon of glycerol. Thus, the structure of **2** was determined as β-(7′Z,10′Z,13′Z)-hexadecatrienoic acid monoglyceride (β-16:3 monoglyceride), as shown in Figure 1, and this was



2

Figure 1. HMBC correlations for compound **2**.

substantiated by analysis of the COSY data (Figure S13, Supporting Information). Assignments of the ^1H and ^{13}C NMR data of **2** are given in Table 1 together with those of **1** for comparison.

Compounds **3** and **4** were identified as α-(9′Z,12′Z,15′Z)-octadecatrienoic acid monoglyceride (α-18:3 monoglyceride) and β-(9′Z,12′Z,15′Z)-octadecatrienoic acid monoglyceride (β-18:3 monoglyceride), respectively, due to good agreement with the spectroscopic data reported.[9] However, the absolute configuration of the C-2 position of the glycerol moiety in **3** was not determined earlier. To determine the absolute configuration, (2S)-α-(9′Z,12′Z,15′Z)-octadecatrienoic acid monoglyceride (**3a**, Scheme S2, Supporting Information) and (2R)-α-(9′Z,12′Z,15′Z)-octadecatrienoic acid monoglyceride (**3b**, Scheme S3, Supporting Information) were synthesized according to the above-mentioned method[9] and showed optical rotation values of $[\alpha]^{26}_D$ +3.8 (*c* 0.42, MeOH) and −3.9 (*c* 0.19, MeOH), respectively. Since naturally occurring **3** exhibited a reported optical rotation of $[\alpha]^{26}_D$ +2.8 (*c* 1.5, MeOH), the absolute configuration of the C-2 position of **3** was determined to be *S*, having an optical purity of 74%, as determined using the same procedure as compound **1** (Figures S14–S17, Supporting Information).

To uncover the biological roles of **1**–**4**, methods for their qualitative and quantitative analysis were required. Deuterium-labeled compounds **8** and **9** were synthesized using [$^2H_2$-1,$^2H_1$-2,$^2H_2$-3]propane-1,2,3-triol. The ionic optimizations for each compound and UPLC conditions to separate the compounds were established, although complete separations between **1** and **2** as well as between **3** and **4** were not performed (Figure 2). This method was applied to reveal a more widely ranging distribution of **1**–**4** among several winter plants (Figure 3). The results also revealed that the endogenous amount of each compound was dependent on the variety of the plant.

It is generally accepted that gibberellic acid promotes bolting in plants. Thus, a possible biological role of **1**–**4** could result from an antagonistic effect on GA. Since the biological importance of GA for developing roots was reported,[10] the inhibitory activities of root elongation using *Arabidopsis thaliana* have been examined, and the results are shown in Figure 4. The application of compounds **1**–**4** at a concentration of 25 μM showed inhibitory effects against the root growth of *A. thaliana* seedlings, in which **1** showed the highest inhibitory effect, causing significant differences in root elongation (Figure 4A). Differences in the biological activity due to the absolute

Table 1. NMR Spectroscopic Data of Compounds 1 and 2 (500 MHz, $CDCl_3$)

	1		2	
position	δ_C, type	δ_H (*J* in Hz)	δ_C, type	δ_H (*J* in Hz)
1	65.7, CH_2	4.16, dd (11.6, 5.9)	62.6, CH_2[a]	3.81, d (4.6)
		4.11, dd (11.6, 5.9)		
2	70.4, CH	3.91, qui (4.9)	75.2, CH	4.90, tt (4.6, 4.6)
3	63.5, CH_2	3.69, dd (11.6, 5.4)	62.6, CH_2[a]	3.81, d (4.6)
		3.59, dd (11.6, 5.4)		
1′	174.4, C		174.1, C	
2′	34.2, CH_2	2.44, t (7.6)	34.4, CH_2	2.37, t (7.3)
3′	25.0, CH_2	2.03, t (7.6)	25.0, CH_2	1.63, qua (7.3)
4′	28.9, CH_2	1.33, m	28.9, CH_2	1.35, t (3.2)
5′	29.4, CH_2	1.33, m	29.4, CH_2	1.35, t (3.2)
6′	27.2, CH_2	2.07, qua (7.6)	27.1, CH_2	2.05, qua (6.5)
7′	130.1, CH	5.38–5.30, m	130.0, CH	5.42–5.24, m
8′	127.2, CH	5.38–5.30, m	127.2, CH	5.42–5.24, m
9′	25.8, CH_2[b]	2.78, t (5.9)	25.8, CH_2[d]	2.78, t (5.7)
10′	128.5, CH[c]	5.38–5.30, m	128.5, CH[e]	5.42–5.24, m
11′	128.3, CH[c]	5.38–5.30, m	128.3, CH[e]	5.42–5.24, m
12′	25.7, CH_2[b]	2.78, t (5.9)	25.7, CH_2[d]	2.78, t (5.7)
13′	128.2, CH	5.38–5.30, m	128.2, CH	5.42–5.24, m
14′	132.2, CH	5.38–5.30, m	132.2, CH	5.42–5.24, m
15′	20.7, CH_2	2.07, qua (7.6)	20.6, CH_2	2.05, qua (6.5)
16′	14.4, CH_3	0.95, t (7.6)	14.4, CH_3	0.95, t (7.6)

[a−e]Interchangeable in each symbol.

DOI: 10.1021/acs.jnatprod.6b00746
J. Nat. Prod. XXXX, XXX, XXX−XXX

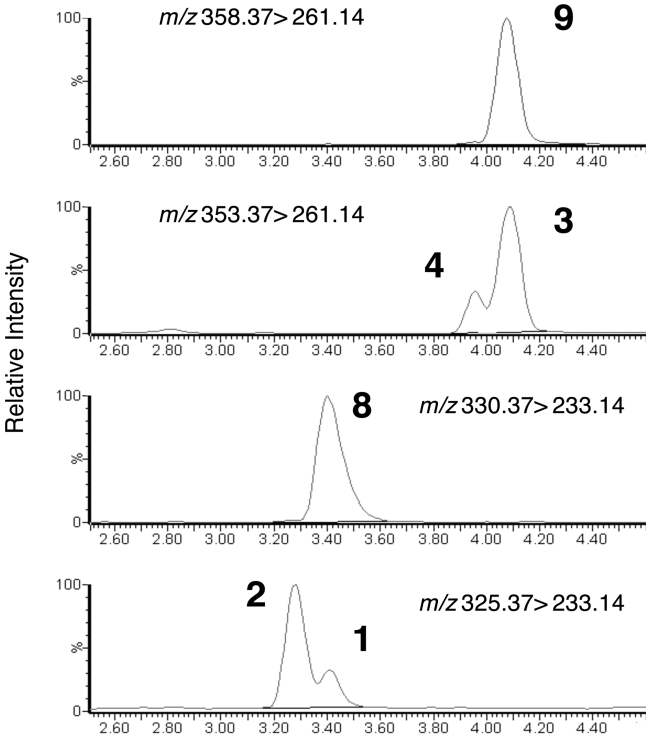


Figure 2. Representative UPLC MS/MS features of the detection of **1**–**4** derived from radish. Compounds **8** and **9** represent the deuterium-labeled compounds of **1** and **3** (internal standard).

configuration at the C-2 position in the monoglyceride moiety were examined, and it was found that the absolute configuration of the compounds did not change the inhibitory effect (Figure 4B and C). It has been reported that jasmonic acid (JA) possesses antibolting and anti-GA activities,[11,12] and thus, it is reasonable to hypothesize that **1a** and **3a** may be able to produce (7*Z*,10*Z*,13*Z*)-hexadecatrienoic acid and α-linolenic acid, respectively, resulting in the generation of jasmonic acid and jasmonoyl isoleucine (JA-Ile), which have shown biological activities via coronatin-insensitive 1 (COI1) protein.[13−16] (−)-JA-L-Ile (25 μM) and **1a** (25 μM) were applied to *coi1-16s A. thaliana* seedlings that had received a disrupted *coronatin-insensitive 1* gene,[17] and the resulting activities were observed, which revealed that **1a** showed biological activity independent of COI1, but (−)-JA-L-Ile needed COI1 to show activity (Figure 4D). Several investigators have used (−)-JA-L-Ile at a 50 μM concentration to check its inhibitory effect on *A. thaliana* seedlings, and it was determined that compound **1** and its related analogues **2**–**4** exhibited activity at 25 and 50 μM concentrations. Thus, it might be possible to categorize these compounds as representative of a new type of plant growth inhibitor, such as those that act by regulating GA activity. Further studies should be performed, such as those examining the organ distribution, biosynthetic pathways, and transportation of the compounds as well as target proteins to suppress GA activity.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were obtained with a JASCO P-2200 polarimeter. NMR spectra were recorded in $CDCl_3$ and benzene-d_6, using a JNM-EX 270 FT-NMR spectrometer (JEOL) [^{1}H NMR: 270 MHz, ^{13}C NMR: 67.5 MHz] and AMX 500 (Bruker) [^{1}H NMR: 500 MHz, ^{13}C NMR: 126 MHz]. FDMS and FIMS analyses were performed on a JMS-T100GCV (JEOL) instrument, and CIMS was performed on a JMS-SX102A (JEOL) instrument. Ultraperformance liquid chromatography (UPLC) was performed on a Waters ACQUITY UPLC system (Waters), which was equipped with a binary solvent delivery manager and a sample manager. MS was performed on a Waters Micromass Quattro Premier Tandem Quadrupole mass spectrometer or a Waters

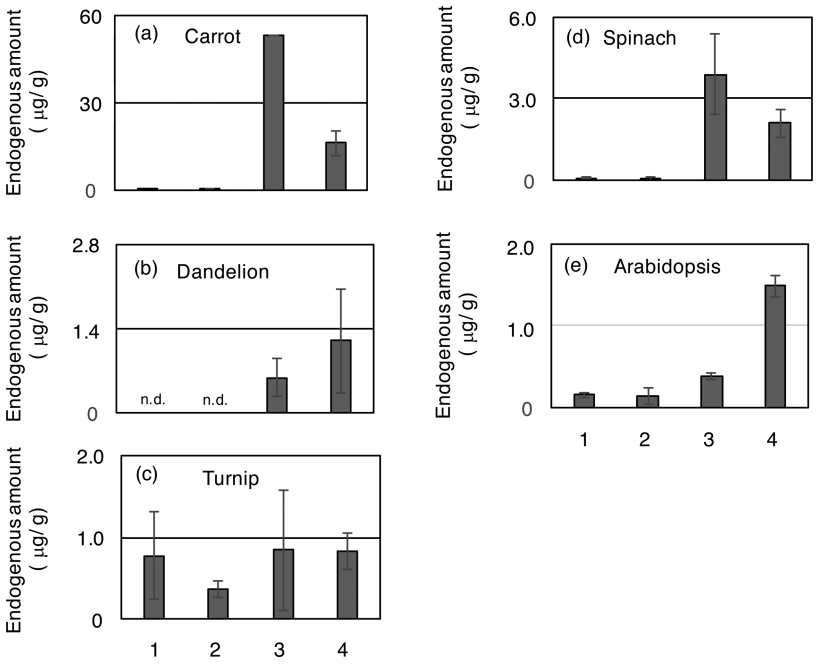


Figure 3. Broader range of distribution of **1**–**4** in annual winter plants. (a) Carrot seeds were sown, and the plants grown in a greenhouse for 3 weeks in early May. (b) Wild plants were harvested in early April in Sapporo, Hokkaido. (c and d) Plants were purchased in a marketplace. (e) Seeds of *A. thaliana* were sown, and plants were grown in a greenhouse for 3 weeks in early May. The leaves were harvested and extracted for analysis of **1**–**4** using UPLC MS/MS. n.d.: not detected.

DOI: 10.1021/acs.jnatprod.6b00746
J. Nat. Prod. XXXX, XXX, XXX−XXX

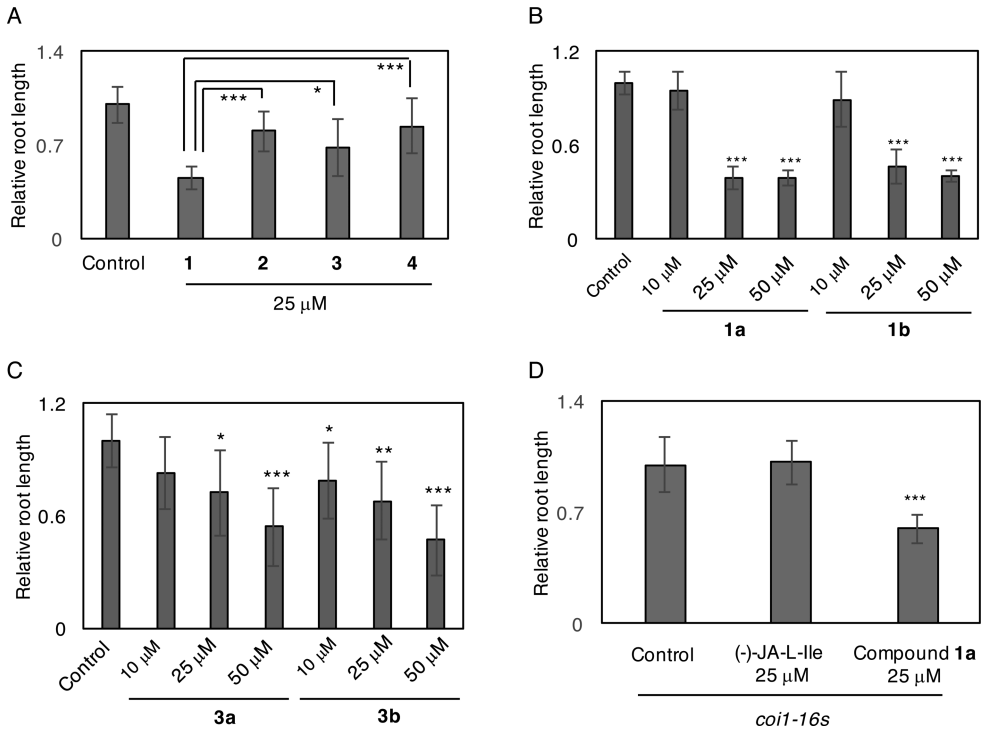


Figure 4. Inhibitory effects of **1**−**4** on root growth of *A. thaliana*.

LCT-Premier Time of Flight (Tof) mass spectrometer. UPLC/MS system control was by MassLynx 4.0.

Plant Material. The leaves of *Raphanus sativus* L. (Brassicaceae) cv. Kitano Haruichi Daikon radish seeds were purchased from Daigaku Noen Co., Ltd., Japan. The radish cultivar was grown in a greenhouse of the Faculty of Agriculture, Hokkaido University, Hokkaido, Japan, in June 2015. The sowing date was May 2, and the leaves were harvested approximately 40 days after sowing. The leaves of *Daucus carota* subsp. *sativus* cv. Kohyoh Nigoh carrot seeds were purchased from Takii & Co., Ltd., Japan. The carrot cultivar was grown in a greenhouse of the Faculty of Agriculture, Hokkaido University, Hokkaido, Japan, in May 2016. The sowing date was April 25, and the leaves were harvested approximately 3 weeks after sowing. The leaves of turnip (*Brassica rapa* subsp. *rapa*) and spinach (*Spinacia oleracea*) were purchased in a supermarket in Hokkaido, Japan, in April 2016. The upper plant of *Taraxacum officinale* was collected from Hokkaido University in Hokkaido, Japan, in April 2016. The plant was identified by one of the authors (H.M.) at the Laboratory of Natural Product Chemistry, Japan, and a voucher specimen (CK.E. 13712) was deposited at the Laboratory of Natural Product Chemistry.

Extraction and Isolation. The harvested leaves (2 kg) were extracted with EtOH (5 L) and then partitioned between EtOAc (500 mL) and H_2O (500 mL). The EtOAc layer was washed with 1 M HCl (500 mL) and saturated aqueous $NaHCO_3$ (500 mL), and the organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to give a residue, which was purified by silica gel flash chromatography with EtOAc−*n*-hexane (3:2), Sephadex LH-20 MeOH−$CHCl_3$ (1:1), and HPLC [YMC Pack ODS-AM, Ⓘ 10 × 300 mm, 2 mL/min, 210 nm, MeOH−H_2O (9:1)] to afford **1** (10.8 mg), **2** (8.7 mg), **3** (21 mg), and **4** (6.9 mg).

*(2S)-α-(7′Z,10′Z,13′Z)-Hexadecatrienoic acid monoglyceride (**1**):* $[\alpha]^{26}_D$ +2.4 (*c* 0.82, MeOH); ^{1}H NMR (270 MHz, $CDCl_3$) and ^{13}C NMR (67.8 MHz, $CDCl_3$) see Table 1; FDMS *m/z* 324 $[M]^+$ (100), 325 $[M + H]^+$ (34.9); HRFDMS *m/z* 324.22859 (calcd for $C_{19}H_{32}O_4$, 324.23006).

*β-(7′Z,10′Z,13′Z)-Hexadecatrienoic acid monoglyceride (**2**):* ^{1}H NMR (270 MHz, $CDCl_3$) and ^{13}C NMR (67.8 MHz, $CDCl_3$) see Table 1; FDMS *m/z* 324 $[M]^+$ (100), 325 $[M + H]^+$ (32.1); HRFDMS *m/z* 324.22860 (calcd for $C_{19}H_{32}O_4$, 324.23006).

*(2S)-α-(9′Z,12′Z,15′Z)-Octadecatrienoic acid monoglyceride (**3**):* $[\alpha]^{27}_D$ +2.8 (*c* 1.5, MeOH); ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.39−5.29 (6H, m), 4.18 (1H, dd, *J* = 11.6, 5.9 Hz), 4.11 (1H, dd, *J* = 11.6, 5.9 Hz), 3.91 (1H, quintet, 4.6), 3.63 (2H, ddd, *J* = 27, 11.6, 5.9 Hz), 2.79 (4H, t, *J* = 5.9 Hz), 2.33 (2H, t, *J* = 7.6 Hz), 2.04 (4H, m), 1.61 (2H, t, *J* = 6.8 Hz), 1.29 (10H, m), 0.95 (3H, t, *J* = 7.6 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 174.4, 132.1, 130.4, 128.4, 128.4, 127.9, 127.3, 70.4, 65.3, 63.9, 34.3, 29.7, 29.3, 29.2, 27.3, 25.8, 25.7, 25.0, 20.7, 14.4; FDMS *m/z* 352 $[M]^+$ (100), 353 $[M + H]^+$ (29.5); HRFDMS *m/z* 352.25962 (calcd for $C_{21}H_{36}O_4$, 352.26136).

*β-(9′Z,12′Z,15′Z)-Octadecatrienoic acid monoglyceride (**4**):* ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.42−5.25 (6H, m), 4.91 (1H, quintet, *J* = 4.9 Hz), 3.81 (4H, t, *J* = 4.6 Hz), 2.79 (4H, t, *J* = 5.4 Hz), 2.36 (2H, t, *J* = 7.6 Hz), 2.15−2.03 (4H, m), 1.62 (2H, m), 1.29 (8H, m), 0.96 (3H, t, *J* = 7.6 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 174.2, 132.1, 130.4, 128.5, 128.4, 127.9, 127.3, 75.2, 62.7, 34.5, 29.7, 29.3, 29.22, 29.21, 27.3, 25.8, 25.9, 25.1, 20.7, 14.4; FDMS *m/z* 352 $[M]^+$ (100), 353 $[M + H]^+$ (29.9); HRFDMS *m/z* 352.25977 (calcd for $C_{21}H_{36}O_4$, 352.26136).

UPLC Tof MS and MS/MS Conditions. The conditions were set according to a previously reported method,[18] except for multiple reaction monitoring (MRM) optimization, which was set up to detect **1**−**4** and for which the conditions are shown in Table S1, Supporting Information.

UPLC Conditions for MS/MS MRM. The UPLC separation was performed on a Waters ACQUITY ethylene-bridged (BEH) C_{18} column (1.7 μm, 2.1 × 100 mm) at 38 °C. The UPLC system was coupled to a Waters Micromass Quattro Premier tandem quadrupole mass spectrometer. The analysis samples were eluted from the column with a mixed solvent of 20% aqueous MeOH with 0.05% AcOH (solvent A) and MeOH with 0.05% AcOH (solvent B), using the linear gradient mode. To examine **1**−**4**, the proportion of A and B was 30:70 from 0 s to 0.2 min, and from 0.2 to 4 min, the proportion of A and B was linearly changed from 30:70 to 0:100. The proportion of 0:100 was maintained from 4 to 5.5 min.

Analysis of Endogenous Amounts of Compounds 1−4. Plant material (2−3 g) was frozen using liquid nitrogen immediately after harvest and then crushed and soaked for 24 h using a solution of EtOH (20 mL) together with the addition of **8** (10 μg/sample) and **9**

DOI: 10.1021/acs.jnatprod.6b00746
J. Nat. Prod. XXXX, XXX, XXX−XXX

(10 μg/sample). The mixture was filtered to give a crude extract, and the volatile components of the extract were removed under reduced pressure to give a residue, which was mixed with a solution of EtOAc (10 mL). The mixture was successively washed with 0.5 M HCl (10 mL) and saturated aqueous $NaHCO_3$ (10 mL), and the organic layer was dried over Na_2SO_4. The volatile components of the organic layer were removed under reduced pressure to give a residue, which was mixed with a solution of EtOAc–*n*-hexane (3:2, 1 mL). The mixture was loaded on a silica gel column, which was eluted into sample tubes (20 mL each) using a solution of EtOAc–*n*-hexane (3:2, 100 mL). The eluates in the second to fourth fractions were mixed, and their volatile components were removed *in vacuo* to give a residue. The residue was dissolved in a mixture of MeOH–H_2O (4:1, 100 μL), and a portion of the solution (5 μL) was subjected to UPLC MS/MS.

Synthesis of Compound 5. The synthesis of **5** was conducted according to a previously reported method,[19] with the exception that 6-bromohexan-1-ol was used as a starting material.

*(7Z,10Z,13Z)-Hexadecatrienoic acid (**5**):* ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.35 (6H, m), 2.78 (4H, t, *J* = 5.4 Hz), 2.33 (2H, t, *J* = 8.1 Hz), 2.05 (5H, m), 1.63 (3H, m), 1.50–1.20 (8H, m), 0.96 (3H, t, *J* = 8.1 Hz); FDMS *m*/*z* 250 $[M]^+$ (100).

General Procedure for the Synthesis of Compounds 1a, 1b, 3a, and 3b. They were synthesized according to a reported method.[20]

*(2S)-α-(7′Z,10′Z,13′Z)-Hexadecatrienoic acid monoglyceride (**1a**):* $[\alpha]^{26}_D$ +4.4 (*c* 0.75, MeOH); HRFDMS *m*/*z* 324.23118 (calcd for $C_{19}H_{32}O_4$, 324.23006).

*(2R)-α-(7′Z,10′Z,13′Z)-Hexadecatrienoic acid monoglyceride (**1b**):* $[\alpha]^{26}_D$ −4.5 (*c* 0.75, MeOH); FDMS *m*/*z* 324 $[M]^+$ (100), 325 $[M + H]^+$ (30.6); HRFDMS *m*/*z* 324.23175 (calcd for $C_{19}H_{32}O_4$, 324.23006).

*(2S)-α-(9′Z,12′Z,15′Z)-Octadecatrienoic acid monoglyceride (**3a**):* $[\alpha]^{27}_D$ +3.8 (*c* 0.42, MeOH); HRFDMS *m*/*z* 352.26028 (calcd for $C_{21}H_{36}O_4$, 352.26136).

*(2R)-α-(9′Z,12′Z,15′Z)-Octadecatrienoic acid monoglyceride (**3b**):* $[\alpha]^{27}_D$ −3.9 (*c* 0.19, MeOH); HRFDMS *m*/*z* 352.26039 (calcd for $C_{21}H_{36}O_4$, 352.26136).

General Procedure of MTPA Esterification. A solution of (*S*)-MTPACl (10 mg, 0.04 mmol) in dry pyridine (0.4 mL) was added to **1** (7.0 mg) in a two-necked flask, and the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was washed with 1 M HCl (30 mL), saturated aqueous $NaHCO_3$ (30 mL), and brine (30 mL), and the organic layer was dried over Na_2SO_4. The volatile components of the organic layer were removed under reduced pressure to give a residue. The residue was purified by silica gel column chromatography with EtOAc–*n*-hexane (1:3) to afford the corresponding product.

*MTPA ester of **1**:* ^{1}H NMR (500 MHz, benzene-d_6) δ 7.68 (2H, d, *J* = 7.9 Hz), 7.14–6.97 (3H, m), 5.55–5.32 (6H, m), 4.13–4.02 (1H, m), 4.02–3.93 (1H, m), 3.92–3.75 (2H, m), 3.59 (1H, d, *J* = 4.4 Hz), 3.41 (3H, s), 2.85 (4H, t, *J* = 5.4 Hz), 2.10–1.92 (6H, m), 1.69 (1H, s), 1.57–1.43 (2H, m), 1.43–1.11 (12H, m), 0.92 (5H, t, *J* = 7.6 Hz); UPLC-Tof MS *m*/*z* 563.2570 $[M + Na]^+$ (calcd for $C_{29}H_{39}O_6F_3Na$, 563.2569).

*MTPA ester of **1a**:* ^{1}H NMR (500 MHz, benzene-d_6) δ 7.67 (2H, d), 7.24–6.94 (3H, m), 5.55–5.31 (6H, m), 4.07 (1H, dd, *J* = 11.3, 6.6 Hz), 3.96 (1H, dd, *J* = 11.3, 4.1 Hz), 3.85 (1H, dd, *J* = 11.7, 4.4 Hz), 3.79 (1H, dd, *J* = 11.8, 5.8 Hz), 3.59 (1H, d, *J* = 5.0 Hz), 3.41 (3H, s), 2.85 (4H, t, *J* = 5.4 Hz), 2.07–1.95 (6H, m), 1.72 (1H, d, *J* = 4.7 Hz), 1.57–1.40 (2H, m), 1.38–1.10 (5H, m), 0.91 (3H, t, *J* = 7.6 Hz); UPLC-Tof MS *m*/*z* 563.2649 $[M + Na]^+$ (calcd for $C_{29}H_{39}O_6F_3Na$, 563.2569).

*MTPA ester of **1b**:* ^{1}H NMR (500 MHz, benzene-d_6) δ 7.68 (2H, d, *J* = 7.6 Hz), 7.07 (3H, dt, *J* = 31.3, 7.4 Hz), 5.51–5.36 (6H, m), 4.13–4.03 (1H, m), 4.02–3.94 (1H, m), 3.91–3.77 (2H, m), 3.60 (1H, s), 3.41 (3H, s), 2.85 (4H, t, *J* = 5.4 Hz), 2.08–1.96 (6H, m), 1.69 (1H, s), 1.54–1.43 (2H, m), 1.41–1.11 (10H, m), 0.92 (3H, t, *J* = 7.6 Hz); UPLC-Tof MS *m*/*z* 563.2645 $[M + Na]^+$ (calcd for $C_{29}H_{39}O_6F_3Na$, 563.2569).

*MTPA ester of **3**:* ^{1}H NMR (500 MHz, benzene-d_6) δ 7.67 (2H, d, *J* = 7.3 Hz), 7.12–6.95 (3H, m), 5.55–5.36 (6H, m), 4.05 (1H, d, *J* = 7.9 Hz), 3.96 (1H, d, *J* = 2.8 Hz), 3.91–3.74 (2H, m), 3.58 (1H, s), 3.41 (3H, s), 2.86 (4H, d, *J* = 15.8 Hz), 2.13–1.95 (6H, m), 1.49 (2H, d, *J* = 6.6 Hz), 1.45–1.07 (23H, m), 0.91 (5H, t, *J* = 7.4 Hz); UPLC-Tof MS *m*/*z* 591.2933 $[M + Na]^+$ (calcd for $C_{31}H_{43}O_6F_3Na$, 591.2909).

*MTPA ester of **3a**:* ^{1}H NMR (500 MHz, benzene-d_6) δ 7.68 (2H, d, *J* = 7.6 Hz), 7.13–6.96 (3H, m), 5.57–5.34 (6H, m), 4.06 (1H, dd, *J* = 11.3, 6.6 Hz), 3.96 (1H, dd, *J* = 11.3, 4.1 Hz), 3.82 (ddd, *J* = 28.6, 11.7, 4.8 Hz), 3.57 (1H, d, *J* = 4.7 Hz), 3.41 (3H, s), 2.95–2.77 (4H, m), 2.14–1.93 (6H, m), 1.66 (1H, d, *J* = 3.8 Hz), 1.50 (2H, t, *J* = 6.8 Hz), 1.41–1.05 (9H, m), 0.91 (3H, t, *J* = 7.6 Hz); UPLC-Tof MS *m*/*z* 591.2896 $[M + Na]^+$ (calcd for $C_{31}H_{43}O_6F_3Na$, 591.2909).

*MTPA ester of **3b**:* ^{1}H NMR (500 MHz, benzene-d_6) δ 7.68 (2H, d, *J* = 7.6 Hz), 7.13–6.96 (3H, m), 5.55–5.36 (6H, m), 4.05 (1H, dd, *J* = 11.0, 3.5 Hz), 3.97 (1H, dd, *J* = 11.5, 5.5 Hz), 3.92–3.77 (2H, m), 3.59 (1H, s), 3.41 (3H, s), 2.95–2.78 (4H, m), 2.10–1.97 (6H, m), 1.65 (1H, s), 1.50 (2H, t, *J* = 6.8 Hz), 1.44–1.06 (17H, m), 0.91 (3H, t, *J* = 7.4 Hz); UPLC-Tof MS *m*/*z* 591.2890 $[M + Na]^+$ (calcd for $C_{31}H_{43}O_6F_3Na$, 591.2909).

Synthesis of Deuterium-Labeled Compounds 8 and 9. Compounds **5** (24.3 mg, 0.1 mmol) and α-linolenic acid (26.0 mg, 0.1 mmol) were prepared. A fatty acid in CH_2Cl_2 (5 mL) was added to a stirred mixture of N,N′-dicyclohexylcarbodiimide (DCC, 34.0 mg, 0.17 mmol), N,N-dimethyl-4-aminopyridine (DMAP, 3.7 mg, 0.03 mmol), and glycerol-1,1,2,3,3-d_5 (28.3 mg, Sigma-Aldrich, St. Louis, MO, USA), and the reaction mixture was further stirred at room temperature for 24 h. The usual workup was employed to give a crude material, which was purified using silica gel column chromatography with EtOAc–*n*-hexane (3:2) and HPLC [YMC Pack ODS-AM, f 10 × 300 mm, 2 mL/min, 210 nm, MeOH–H_2O (9:1)] to afford compounds **8** (4.2 mg, 0.013 mmol) and **9** (8.0 mg, 0.022 mmol).

*[$^2H_2$-1,$^2H_1$-2,$^2H_2$-3]-α-(7′Z,10′Z,13′Z)-Hexadecatrienoic acid monoglyceride (**8**):* HRFDMS *m*/*z* 329.26269 (calcd for $C_{19}H_{27}D_5O_4$, 329.26144).

*[$^2H_2$-1,$^2H_1$-2,$^2H_2$-3]-α-(9′Z,12′Z,15′Z)-Octadecatrienoic acid monoglyceride (**9**):* HRFDMS *m*/*z* 357.29315 (calcd for $C_{21}H_{31}D_5O_4$, 357.29274).

Root Growth Inhibition Assay. The root growth inhibitory test was performed according to a reported method.[21]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.6b00746.

Representative HPLC features for purification and representative UPLC MS/MS features for compounds **1**–**4**; FDMS, ^{1}H NMR, ^{13}C NMR, DEPT, COSY, HSQC, and HMBC spectra for **2**; ^{1}H NMR spectra for each (*S*)-MTPA ester; synthetic Schemes S1–S3 for **1b**, **3a**, **3b**; and conditions of MS optimization for multiple reaction monitoring in the positive mode for compounds **1**, **3**, **8**, and **9** (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Tel: + 81 11 706 2495. Fax: + 81 11 706 2505. E-mail: matsuura@chem.agr.hokudai.ac.jp.

ORCID

Hideyuki Matsuura: 0000-0002-9605-7188

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors would like to thank Mr. Y. Takata and Dr. E. Fukushi (Faculty of Agriculture, Hokkaido University) for

DOI: 10.1021/acs.jnatprod.6b00746
J. Nat. Prod. XXXX, XXX, XXX–XXX

assistance in obtaining the spectroscopic data. We would also like to thank the Research Faculty of Agriculture, Hokkaido University, for the use of the UPLC MS/MS system.

REFERENCES

(1) Engelen Eigles, G.; Erwin, J. E. *Sci. Hortic.* **1997**, *70*, 197−202.
(2) Yoo, K. C.; Uemoto, S. *Plant Cell Physiol.* **1976**, *17*, 863−865.
(3) Nishijima, T.; Katsura, N.; Koshioka, M.; Yamazaki, H.; Nakayama, M.; Yamane, H.; Yamaguchi, I.; Yokota, T.; Murofushi, N.; Takahashi, N.; Nonaka, M.; Mander, L. N. *J. Jpn. Soc. Hortic. Sci.* **1998**, *67*, 319−324.
(4) Chailakh, M. K. *Annu. Rev. Plant Physiol.* **1968**, *19*, 1−36.
(5) Suge, H.; Rappapor, L. *Plant Physiol.* **1968**, *43*, 1208−1214.
(6) Nishijima, T.; Katsura, N.; Koshioka, M.; Yamazaki, H.; Mander, L. N. *Plant Growth Regul.* **1997**, *21*, 207−214.
(7) Yoshida, Y.; Takada, N.; Koda, Y. *Plant Cell Physiol.* **2010**, *51*, 1341−1349.
(8) Hungerford, N. L.; Kitching, W. *J. Chem. Soc., Perkin Trans. 1* **1998**, 1839−1858.
(9) Katoch, R.; Trivedi, G. K.; Phadke, R. S. *Bioorg. Med. Chem.* **1999**, *7*, 2753−2758.
(10) Shani, E.; Weinstain, R.; Zhang, Y.; Castillejo, C.; Kaiserli, E.; Chory, J.; Tsien, R. Y.; Estelle, M. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 4834−4839.
(11) Koda, Y.; Ohkawa-Takahashi, K.; Kikuta, Y. *Plant Prod. Sci.* **2001**, *4*, 131−135.
(12) Yang, D. L.; Yao, J.; Mei, C. S.; Tong, X. H.; Zeng, L. J.; Li, Q.; Xiao, L. T.; Sun, T. P.; Li, J. G.; Deng, X. W.; Lee, C. M.; Thomashow, M. F.; Yang, Y. N.; He, Z. H.; He, S. Y. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, E1192−E1200.
(13) Thines, B.; Katsir, L.; Melotto, M.; Niu, Y.; Mandaokar, A.; Liu, G.; Nomura, K.; He, S.; Howe, G.; Browse, J. *Nature* **2007**, *448*, 661−665.
(14) Chini, A.; Fonseca, S.; Fernández, G.; Adie, B.; Chico, J.; Lorenzo, O.; García-Casado, G.; López-Vidriero, I.; Lozano, F.; Ponce, M.; Micol, J.; Solano, R. *Nature* **2007**, *448*, 666−671.
(15) Katsir, L.; Schilmiller, A.; Staswick, P.; He, S.; Howe, G. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 7100−7105.
(16) Chini, A.; Boter, M.; Solano, R. *FEBS J.* **2009**, *276*, 4682−4692.
(17) Chen, J.; Sonobe, K.; Ogawa, N.; Masuda, S.; Nagatani, A.; Kobayashi, Y.; Ohta, H. *J. Plant Res.* **2013**, *126*, 161−168.
(18) Sato, C.; Seto, Y.; Nabeta, K.; Matsuura, H. *Biosci., Biotechnol., Biochem.* **2009**, *73*, 1962−1970.
(19) Hungerford, N. L.; Kitching, W. *J. Chem. Soc., Perkin Trans. 1* **1998**, 1839−1858.
(20) Katoch, R.; Trivedi, G. K.; Phadke, R. S. *Bioorg. Med. Chem.* **1999**, *7*, 2753−2758.
(21) Kitaoka, N.; Kawaide, H.; Amano, N.; Matsubara, T.; Nabeta, K.; Takahashi, K.; Matsuura, H. *Phytochemistry* **2014**, *99*, 6−13.

DOI: 10.1021/acs.jnatprod.6b00746
J. Nat. Prod. XXXX, XXX, XXX−XXX