Oxylipins Arabidopsides C and D from *Arabidopsis thaliana*

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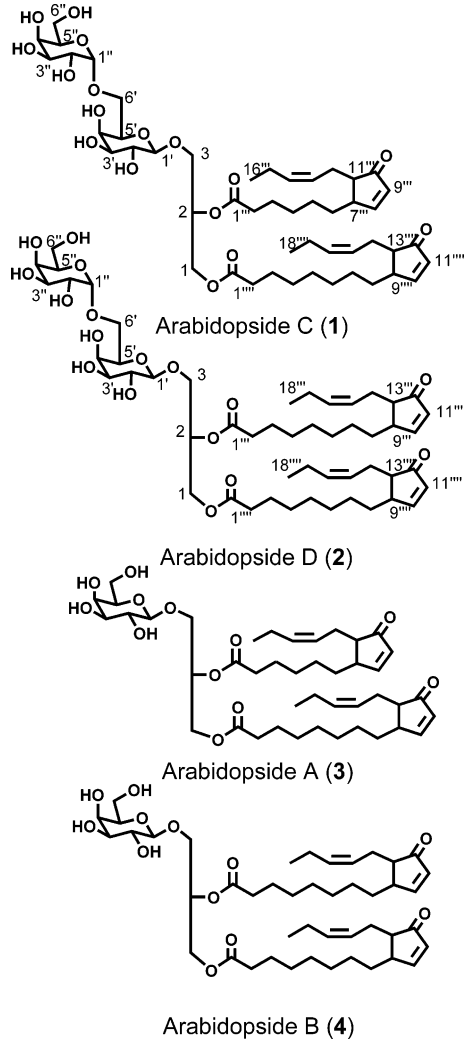
Two new oxylipins, arabidopsides C (**1**) and D (**2**), were isolated from the aerial parts of *Arabidopsis thaliana*, and the structures of **1** and **2** were elucidated using spectroscopic data, primarily NMR and MS, and chemical means. Arabidopsides C (**1**) and D (**2**) are rare digalactosyl diacylglycerides containing 12-oxophytodienoic acid and/or dinor-oxophytodienoic acid. Arabidopside D (**2**) and arabidopsides A (**3**) and B (**4**), which were also isolated from this plant, exhibited inhibitory effects on the growth of the root of cress (*Lepidium sativum*) seedlings at 5×10^{-5} mol/L.

Arabidopsis thaliana ecotype col-0 (Brassicaceae) is known as a model plant, and biologists have used this plant for various genetic studies. However, few examples of bioactive substances from *A. thaliana* have been reported, except for some phytohormones. We previously isolated two unique oxylipins, arabidopsides A (**3**) and B (**4**), from the aerial parts of *A. thaliana*.[1] Compounds **3** and **4** are rare monogalactosyl diacylglycerides containing 12-oxophytodienoic acid (OPDA) and/or dinor-oxophytodienoic acid (dn-OPDA), which are known as precursors of jasmonic acid (JA).[2,3] JA and OPDA have received much attention because they play important roles in regulation of developmental and defense gene expression of plants.[4] Further examination of an extract of *A. thaliana* resulted in isolation of two additional oxylipins, arabidopsides C (**1**) and D (**2**), which are new digalactosyl diacylglycerides containing OPDA and/or dn-OPDA. This paper describes the isolation and structure elucidation of **1** and **2** and their inhibitory effect on cress root growth.

The aerial parts (100 g) of *A. thaliana* were extracted with MeOH. The MeOH extract was partitioned between EtOAc and H_2O. The EtOAc-soluble portion was subjected to a silica gel column ($CHCl_3$/MeOH, 1:1) to afford a glycolipid fraction, which was fractionated by reversed-phase C_{18} HPLC (CH_3CN/H_2O, 1:1) to give arabidopsides C (**1**, 0.0004%, wet weight) and D (**2**, 0.0018%) as colorless amorphous solids, together with arabidopsides A (**3**, 0.0040%) and B (**4**, 0.0018%).[1]

The molecular formula, $C_{51}H_{80}O_{17}$, of arabidopside D (**2**) was established by HRESIMS [*m/z* 987.5285 $(M + Na)^+$, Δ −0.8 mmu]. The IR spectrum implied the presence of hydroxy (3424 cm^{-1}), ester carbonyl (1736 cm^{-1}), and unsaturated carbonyl (1714 and 1631 cm^{-1}) groups. The gross structure of **2** was deduced from detailed analysis of the ^1H and ^{13}C NMR data (Table 1) aided by 2D NMR experiments ($^1H-^1H$ COSY, HMQC, and HMBC). The ^1H and ^{13}C NMR data of **2** were similar to those of arabidopside B (**4**) except for signals due to the sugar moieties. The $^1H-^1H$ COSY connectivities of C-1 through C-3, C-1′ through C-6′, and C-1″ through C-6″ indicated the presence of a glycerol and two sugar components. The two sugars were assigned to be galactoses by NOESY correlations of



H-1′ to H-3′, H-4′ to H-3′ and H-5′, H-1″ to H-3″, and H-4″ to H-3″ and H-5″ and the $^1H-^1H$ coupling constants (Table 1). The anomeric proton (H-1′, δ_H 4.29) of **2** showed HMBC cross-peaks with C-3 (δ_C 68.6) and C-2′ (δ_C 73.1), and the other anomeric proton (H-1″, δ_H 4.77) with C-6′ (δ_C 68.6) and C-2″ (δ_C 71.0). The ^1H and ^{13}C NMR spectroscopic data and $^1H-^{13}C$ coupling constants obtained by a nondecoupled HSQC experiment of **2** in CD_3OD (C-1′, J_{CH} = 169.8 Hz; C-1″, J_{CH} = 162.8 Hz) revealed that **2** possessed the 1,2-

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Table 1. ^{13}C and ^{1}H NMR Assignments (δ) for the Glycerol and Sugar Moieties of **1** and **2**

	arabidopside C (**1**)			arabidopside D (**2**)		
	$^{13}C^{a}$	$^{1}H^{b}$		$^{13}C^{a}$	$^{1}H^{b}$	
1	64.5	4.44	(dd, 5.4, 10.8)	64.9	4.26	(dd, 6.6, 12.0)
		4.24	m		4.47	(dd, 2.4, 12.0)
2	71.1	5.29	m	71.4	5.29	m
3	67.9	4.00	m	68.6	3.98	(dd 5.4, 10.8)
		3.76	m		3.76	m
1′	106.1	4.27	(d, 7.2)	106.0	4.29	(d, 7.2)
2′	73.2	3.55	(dd, 7.2, 14.1)	73.1	3.53	(dd, 7.8, 12.6)
3′	75.6	3.51	(dd, 4.2, 14.1)	75.5	3.52	(dd, 2.4, 10.2)
4′	71.0	3.92	(d, 4.2)	71.0	3.92	(d, 2.4)
5′	75.5	3.78	m	75.4	3.78	m
6′	67.1	3.96	m	68.6	3.72	(dd, 4.8, 11.4)
		3.71	m		3.93	m
1″	101.4	4.91	(d, 3.2)	101.2	4.77	(d, 3.6)
2″	70.8	3.82	(dd, 3.2, 10.2)	71.0	3.83	(dd, 3.6, 10.2)
3″	72.2	3.76	(dd, 3.3, 10.2)	72.1	3.77	(dd, 3.0, 10.2)
4″	71.9	3.94	(d, 3.3)	71.8	3.94	(d, 3.0)
5″	73.4	3.88	m	73.3	3.89	m
6″	62.8	3.76	m	63.5	3.76	m
		3.76	m		3.76	m

[a] 600 MHz; CD_3OD (δ 49.8). [b] 600 MHz; CD_3OD (δ 3.35).

di-*O*-acyl-3-*O*-[α-D-galactopyranosyl-(1″→6′)-*O*-β-D-galactosyl]-*sn*-glycerol moiety.[5] The $^{1}H-^{1}H$ COSY connectivities of C-9‴ (or C-9⁗) through C-11‴ (or C-11⁗) and C-9‴ (or C-9⁗) through C-13‴ (or C-13⁗) and HMBC correlations of H-11‴ (or H-11⁗) (δ_C 6.21) to C-9‴ (or C-9⁗) (δ_C 44.8), C-12‴ (or C-12⁗) (δ_C 215.2), and C-13‴ (or C-13⁗) (δ_C 49.8) indicated the presence of two cyclopentenone moieties. The $^{1}H-^{1}H$ COSY connectivities of C-13‴ (or C-13⁗) through C-18‴ (or C-18⁗) and HMBC correlations of Ha-14‴ (or Ha-14⁗) (δ_H 2.50) and Hb-14‴ (or Hb-14⁗) (δ_H 2.24) to C-15‴ (or C-15⁗) (δ_C 126.9) and C-16‴ (or C-16⁗) (δ_C 135.7) revealed that 2-penetene groups connected to C-13‴ and C-13⁗. *Z*-Geometries of two disubstituted double bonds at C-15‴−C-16‴ and C-15⁗−C-16⁗ were deduced from the carbon chemical shifts of allylic carbons (C-14‴ or C-14⁗, δ_C 24.7; C-17‴ or C-17⁗, δ_C 22.3).[6] These data and proton and carbon resonances indicated that **2** possessed two *cis*-12-oxophytodienoic acids (OPDA). HMBC correlations of Ha-1 and Hb-1 to an ester carbonyl carbon (δ_C 176.8) and chemical shifts (δ_H 5.29; δ_C 71.4) of C-2 indicated that the OPDA connected to C-1 and C-2. The chiral GC analysis of methanolisates of **2** after treatment with HCl/MeOH detected the mixture (10:3) of (9*S*,13*S*)-**5** and (9*R*,13*R*)-**5** (Figure 1).[7] On the other hand, the dibenzyl glycerol (**6**) derived from **2** was racemic by chiral GC analysis (Figure 1).[8]

Arabidopside C (**1**) showed a pseudomolecular ion peak at *m/z* 959 $(M + Na)^+$ in the ESIMS. HRESIMS analysis revealed the molecular formula of **1** to be $C_{49}H_{76}O_{17}$ [*m/z* 959.4971 $(M + Na)^+$, Δ −0.9 mmu], indicating that **1** was an ethylene homologue of **2**. The ^{1}H and ^{13}C NMR spectra of **1** were similar to those of **2** except for the signals due to the methylene chains. The two lipids were presumed to be OPDA and dn-OPDA moieties judging from spectral data of OPDA and dn-OPDA in arabidopside A (**3**).[1] HMBC correlations of Ha-1 (δ_H 4.44) and Hb-1 (δ_H 4.24) to the ester carbonyl carbon (δ_C 175.1) and chemical shifts (δ_H 5.29; δ_C 71.1) of C-2 indicated that OPDA and dn-OPDA connected to C-1 and C-2. To define the locations of these lipids in the β-galactosylglycerol moiety of **1**, we applied enzymatic hydrolysis with lipase type XI (Sigma) to preferentially hydrolyze *sn*1 fatty acids.[9] The lipase type XI-catalyzed hydrolysis of **1** afforded OPDA (**7**, Figure 1), which was identified as OPDA methyl ester derived from the hydrolysate with trimethylsilyldiazomethane,[10] using GC analysis.

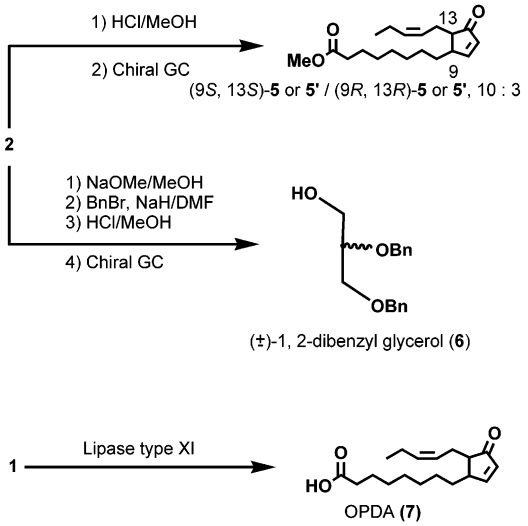

Figure 1. Derivatization of **1** and **2**.

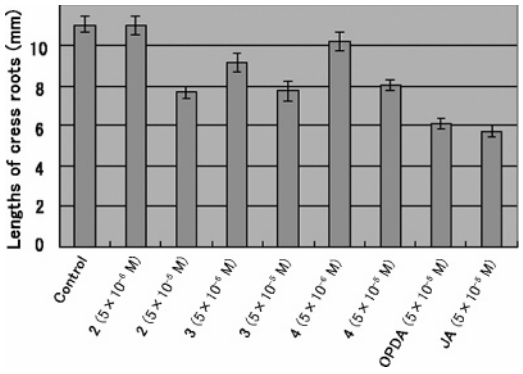

Figure 2. Growth inhibitory effects of arabidopsides A (**3**), B (**4**), and D (**2**).

Therefore, arabidopside C (**1**) was assigned to be *sn*1-*O*-(12-oxophytodienoyl)-*sn*2-*O*-(dinor-oxophytodienoyl)digalactosyl diglyceride.

As JA showed inhibitory effects on root growth of various plants,[4] the effects of arabidopsides A (**3**), B (**4**), and D (**2**) were also examined by the application to cress seeds. Ten seeds of cress (*Lepidium sativum* L.) were placed on a filter paper moistened with test solution and kept for 40 h at 24 °C in the dark, after which the lengths of their roots were measured. As shown in Figure 2, arabidopsides A (**3**), B (**4**), and D (**2**) inhibited 30% of the growth of cress roots at 5×10^{-5} mol/L, while OPDA and JA inhibited root growth 50% at 5×10^{-5} mol/L.

Arabidopsides C (**1**) and D (**2**) are the first digalactosyl diacylglycerides containing OPDA and/or dn-OPDA, while arabidopsides A (**3**) and B (**4**) and MGDG-O are monogalactosyl diacylglycerides containing OPDA and/or dn-OPDA.[1,11] Arabidopsides A (**3**), B (**4**), and D (**2**) exhibited inhibitory effects on the growth of cress roots at 5×10^{-5} mol/L, suggesting that the sugar moiety has little effect on growth activity.

Experimental Section

General Experimental 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 on a Varian Unity INOVA 600 spectrometer in CD_3OD. The resonances of CD_3OD at δ_H 3.35 ppm and δ_C 49.8 ppm were used as internal references for NMR spectra. ESIMS were recorded on a micromass Q-Tof-2 mass spectrometer.

Plant Material. The seeds of *Arabidopsis thaliana* ecotype col-0 (Brassicaceae) were purchased from Lehle Seeds. The seeds were immersed in H_2O at 4 °C for 2 days before sowing on rock wool (rock fiber, NITTOBO, Japan). They were then cultured under continuous light (24 h, ca. 3800 lux) at 24 °C, until forming a flower bud.

Extraction and Isolation. Aerial parts of *A. thaliana* (100 g) were extracted with MeOH (1 L × 2) and evaporated to dryness in vacuo at 30 °C. The MeOH extract (3.66 g) was then partitioned between EtOAc (100 mL × 3) and H_2O (100 mL). The EtOAc-soluble portion (0.9 g) was subjected to a silica gel column (1.1 × 31 cm, $CHCl_3$/MeOH, 19:1 → 1:1). The fraction eluted with $CHCl_3$/MeOH, 1:1, was applied to a C_{18} Sep-Pak cartridge (CH_3CN/H_2O, 3:1, Waters), and the fraction (10.3 mg) containing arabidopsides C (**1**) and D (**2**) was further separated by reversed-phase HPLC [Deverosil ODS HG-5 (Nomura Chemical, ϕ 1.0 × 25 cm), flow rate 2.5 mL/min; solvent CH_3CN/H_2O (11:9); detection UV (222 nm)] to give arabidopsides C (**1**, 1.8 mg, t_R 22 min) and D (**2**, 0.4 mg, t_R 38 min). The $CHCl_3$/MeOH, 19:1, fraction was separated by reversed-phase HPLC [Deverosil ODS HG-5, flow rate 2.5 mL/min; solvent CH_3CN-H_2O (4:1); detection UV (222 nm)] to afford arabidopsides A (**3**, 4.0 mg, t_R 10 min) and B (**4**, 1.8 mg, t_R 13 min).

Arabidopsside C (1): colorless amorphous solid; $[\alpha]_D^{24}$ +30.0° (*c* 0.69, MeOH); IR (KBr) ν_{max} 3424, 1736, 1714, and 1631 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ 7.96 (2H, m, H-8‴, H-10⁗), 6.20 (2H, m, H-9‴, H-11⁗), 5.45 (4H, m, H-13‴, H-14‴, H-15⁗, H-16⁗), 3.10 (2H, m, H-7‴, H-9⁗), 2.54 (2H, m, H-11‴, H-13⁗), 2.48 (2H, m, Ha-12‴, Ha-14⁗), 2.39 (4H, m, H_2-2‴, H_2-2⁗), 2.24 (2H, m, Hb-12‴, Hb-14⁗), 2.12 (4H, m, H_2-15‴, H_2-17⁗), 1.83 (2H, m, Ha-8‴, Ha-8⁗), 1.64 (4H, m, H_2-3‴, H_2-3⁗), 1.36 (12H, m, H_2-4‴, H_2-5‴, H_2-4⁗, H_2-5⁗, H_2-6⁗, H_2-7⁗), 1.27 (2H, m, Hb-8‴, Hb-8⁗), and 1.02 (6H, t, J = 7.2 Hz, H_3-16‴, H_3-18⁗); ^{13}C NMR (150 MHz, CD_3OD) δ 213.8 (C-10‴, C-12⁗), 175.1 (C-1‴, C-1⁗), 171.2 (C-8‴, C-10⁗), 135.7 (C-14‴, C-16⁗), 133.1 (C-9‴, C-11⁗), 126.7 (C-13‴, C-15⁗), 51.4 (C-11‴, C-13⁗), 48.8 (C-7‴, C-9⁗), 36.0 (C-2‴, C-2⁗), 35.6 (C-6‴, C-8⁗), 31.6 (C-6⁗), 31.3 (C-4‴, C-4⁗), 31.0 (C-7⁗), 28.9 (C-5‴, C-5⁗), 26.8 (C-3‴, C-3⁗), 24.5 (C-12‴, C-14⁗), 22.8 (C-15‴, C-17⁗), and 15.2 (C-16‴, C-18⁗); ^{13}C and ^1H NMR data for sugars and glycerol moieties, see Table 1; ESIMS (pos.) *m/z* 959 $(M + Na)^+$, HRESIMS (pos.) *m/z* 959.4971 $(M + Na)^+$, calcd for $C_{51}H_{80}O_{17}Na$, 959.4979.

Arabidopsside D (2): colorless amorphous solid; $[\alpha]_D^{22}$ +67.2° (*c* 0.69, MeOH); IR (KBr) ν_{max} 3424, 1736, 1714, and 1631 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ 7.96 (2H, dd, J = 2.1, 5.8 Hz, H-10‴, H-10⁗), 6.21 (2H, dd, J = 2.1, 5.8 Hz, H-11‴, H-11⁗), 5.45 (4H, m, H-15‴, H-16‴, H-15⁗, H-16⁗), 3.09 (2H, m, H-9‴, H-9⁗), 2.54 (2H, m, H-13‴, H-13⁗), 2.50 (2H, m, Ha-14‴, Ha-14⁗), 2.37 (4H, m, H_2-2‴, H_2-2⁗), 2.24 (2H, m, Hb-14‴, Hb-14⁗), 2.13 (4H, m, H_2-17‴, H_2-17⁗), 1.83 (2H, m, Ha-8‴, Ha-8⁗), 1.65 (4H, m, H_2-3‴, H_2-3⁗), 1.36 (16H, m, H_2-4‴, H_2-5‴, H_2-6‴, H_2-7‴, H_2-4⁗, H_2-5⁗, H_2-6⁗, H_2-7⁗), 1.23 (2H, m, Hb-8‴, Hb-8⁗) and 1.02 (6H, t, J = 7.5 Hz, H_3-18‴, H_3-18⁗); ^{13}C NMR (150 MHz, CD_3OD) δ 215.2 (C-12‴, C-12⁗), 176.8 (C-1‴, C-1⁗), 171.2 (C-10‴, C-10⁗), 135.7 (C-16‴, C-16⁗), 134.1 (C-11‴, C-11⁗), 126.9 (C-15‴, C-15⁗), 49.8 (C-13‴, C-13⁗), 44.8 (C-9‴, C-9⁗), 36.0 (C-2‴, C-2⁗), 35.6 (C-8‴, C-8⁗), 31.5 (C-6‴, C-6⁗), 31.3 (C-4‴, C-4⁗), 30.9 (C-7‴, C-7⁗), 29.3 (C-5‴, C-5⁗), 26.8 (C-3‴, C-3⁗), 24.7 (C-14‴, C-14⁗), 22.3 (C-17‴, C-17⁗) and 15.3 (C-18‴, C-18⁗); ^{13}C and ^1H NMR data for sugars and glycerol moieties, see Table 1; ESIMS (pos.) *m/z* 987 $(M + Na)^+$, HRESIMS (pos.) *m/z* 987.5285, calcd for $C_{51}H_{80}O_{17}Na$, 987.5293.

Methanolysis of 2 and Chiral GC Analyses of the Methanolysates. After a solution of **2** (0.1 mg) in 0.6 mol/L HCl/MeOH (0.1 mL) was stirred at 60 °C for 45 min, the solvent was removed under reduced pressure. The residue was partitioned between hexane and 90% MeOH, and the hexane-soluble materials were used for chiral GC analyses.[7] Chiral GC conditions: column: γ-DEX 120 capillary column, SUPELCO; program rate: 40 → 160 °C (at 10 °C/min), 160 °C (360 min), 160 → 200 °C (at 10 °C/min), and 200 °C (60 min). The absolute configurations of the methyl ester of OPDA (OPDAMe, **5**) were determined in comparison to the retention time (min) of OPDAMe derived from OPDA, purchased from Cayman Chemical, by methylation with trimethylsilyldiazomethane.[10] The *cis* enantiomers, (9‴*S*, 13‴*S*)-**5** (t_R 397 min) and (9‴*R*,13‴*R*)-**5** (t_R 397 min), (9⁗*S*,13⁗*S*)-**5′** (t_R 397 min) and (9⁗*R*,13⁗*R*)-**5′** (t_R 397 min), were inseparable. The *trans* enantiomers, (9‴*S*,13‴*R*)-**5** (t_R 347 min) and (9‴*R*,13‴*S*)-**5** (t_R 350 min), (9⁗*S*,13⁗*R*)-**5′** (t_R 347 min) and (9⁗*R*,13⁗*S*)-**5′** (t_R 350 min) (10:3), were produced from *cis* forms by enolization.

1, 2-Dibenzyl Glycerol (6) Derived from 2 and Chiral GC Analysis of 6. This derivatization followed the method of Uzawa.[8] A solution of **2** (1.2 mg) in dry MeOH (0.1 mL) was treated with NaOMe/MeOH (10 equiv) at room temperature for 1 h. The reaction mixture was partitioned between hexane and H_2O, and the H_2O-soluble portion was concentrated under N_2 gas. A solution of the concentrate in dry DMF (1 mL) was treated with NaH at room temperature for 30 min. Benzyl bromide (5 μL) was added to the reaction solution, then the mixture was stirred at room temperature for a further 14 h. The reaction was quenched by the addition of saturated aqueous NH_4Cl and extracted with EtOAc (5 mL) three times. The EtOAc extract was concentrated in vacuo. The residual syrup was subjected to preliminary separation by TLC [benzene/hexane (10:1) → EtOAc], and a solution of the concentrate in 10% dry HCl/MeOH (600 μL) was heated at 100 °C for 8 h. The reaction mixture was poured into EtOH and concentrated under N_2 gas. The reaction mixture (0.19 mg) was subjected to chiral-GC analysis. Chiral-GC conditions: column: CP-cyclodex-β-2,3,6 M-capillary column, Varian; program rate: 40 → 150 °C (at 10 °C/min), 150 °C (450 min), 150 → 200 °C (at 10 °C/min), and 200 °C (60 min). The absolute configuration of the dibenzyl glycerol were determined by the retention time (min) of (*S*)- and (*R*)-dibenzyl glycerol (*S*: 373 min, *R*: 376 min) derived from (*S*)- and (*R*)-glycerol acetonide according to the procedure of Ashton.[12]

Enzymatic Hydrolysis of 1. A solution of **1** (0.6 mg) and Lipase type XI (0.72 units, Sigma) in the presence of Triton X-100 (2.5 mg) in boric acid/borax buffer (0.63 mL, pH 7.7) was stirred at 38 °C for 12 h. The reaction was quenched with AcOH (0.1 mL), and then EtOH (2 mL) was added to the reaction mixture. The solvent was removed under reduced pressure, and the residue was purified using a silica gel column (hexane/EtOAc, 1:1) to yield OPDA (**7**, 0.4 mg, 62%),[9] which was identified as OPDA methyl ester derived from the hydrolysate with trimethylsilyldiazomethane,[10] using GC analysis.

Bioassay. Ten seeds of cress (*Lepidium sativum* L.) were placed on a filter paper (No. 1, Toyo) moistened with 500 μL of test solution containing 0.01% Triton X-100 (v/v) in a 2.7 cm Petri dish and kept for 40 h at 24 °C in the dark, after which the lengths of their roots were measured. Seedlings cultured on the solution containing 0.01% Triton X-100 was used as controls. Data are represented as mean values with standard errors of three experiments.

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