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ARABIDOPSIDE F, A NEW OXYLIPIN FROM *ARABIDOPSIS THALIANA*

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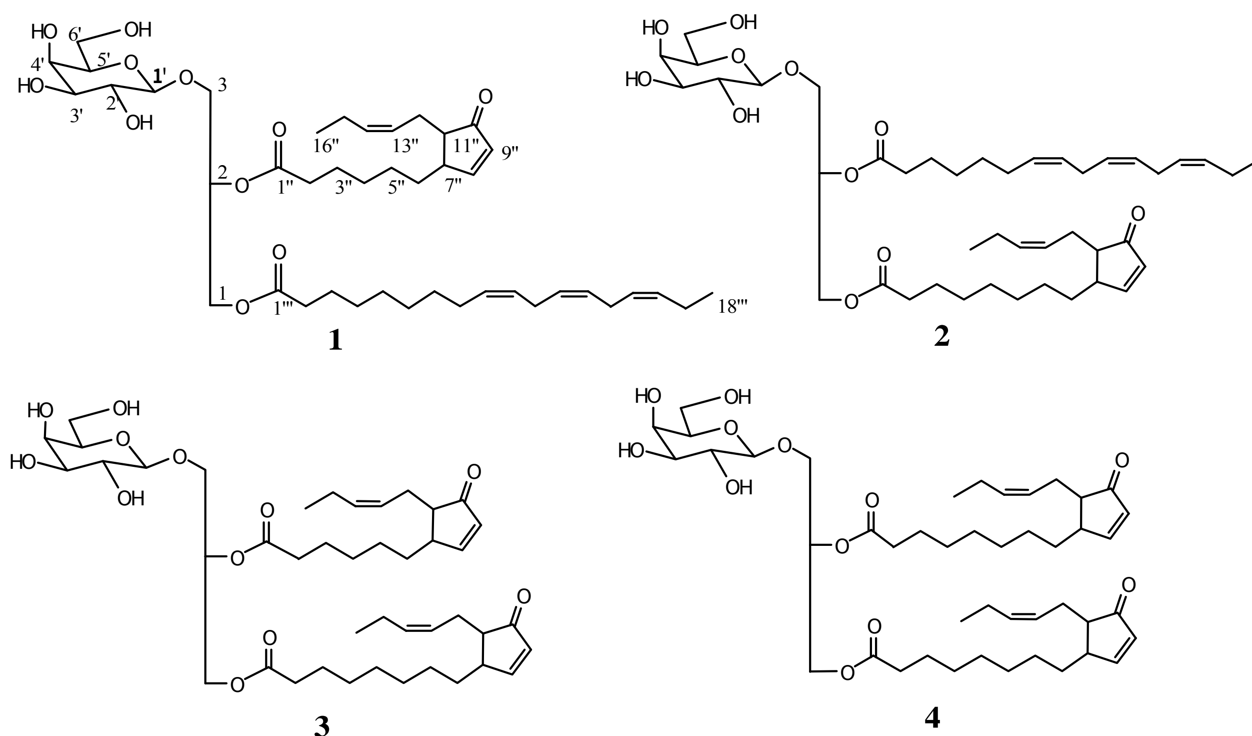
Abstract- A new oxylipin, arabidopside F (**1**) was isolated from the aerial parts of *Arabidopsis thaliana*, together with MGDG-O (**2**), arabidopsides A (**3**) and B (**4**). The structure of arabidopside F (**1**) was elucidated by spectroscopic data and chemical means. Arabidopside F (**1**) was a rare monogalactosyl diacylglyceride containing dinor-oxophytodienoic acid and **1** exhibited inhibitory effects on the growth of the root of cress (*Lepidium sativum* L.) seedlings at 5×10^{-5} mol/L.

Recent studies on oxylipins, which are metabolites derived from oxygenated fatty acid, have clarified that these compounds play important roles in the growth regulations of plants. Jasmonic acid (JA) and methyl jasmonate (MeJA) are most famous oxylipins and they are known to regulate many defense and developmental pathways in plants.[1] In our search for bioactive substances from *A. thaliana*, we previously isolated four oxylipins, named arabidopsides A, B, C, and D, which were monogalactosyl diacylglycerides or digalactosyl diacylglycerides containing 12-oxophytodienoic acid (OPDA) and/or dinor-oxophytodienoic acid (dn-OPDA). OPDA and dn-OPDA were known as precursors of JA[2~5] and have received much attention because they play important roles in regulation of developmental and defense gene expression of plants as JA and MeJA.[6] Recently, arabidopside E, a new monogalactosyldiacylglycerol containing two OPDA and one dn-OPDA acyl chain, was isolated from *A. thaliana* and was shown to inhibit growth of a bacterial pathogen in *vitro*.[7] Further examination of an extract of *A. thaliana* resulted in isolation of an additional new oxylipin, arabidopside F (**1**), which was a monogalactosyl diacylglyceride containing dn-OPDA from the aerial parts of this plant. In this paper we describe the isolation, structure elucidation, and biological activity of arabidopside F (**1**).

Dedicated to celebration of the 70th birthday of Dr. Satoshi Omura



The aerial parts (530 g) of *A. thaliana* ecotype Shokei (Brassicaceae) were extracted with MeOH. The MeOH extracts were partitioned between EtOAc and H_2O. The EtOAc-soluble portions were subjected to a silica gel column ($CHCl_3$/MeOH, 19:1→1:1) and the fraction eluted with $CHCl_3$/MeOH (4:1) was applied to a C_{18} Sep-Pak cartridge (CH_3CN/H_2O, 3:1, Waters) to afford a glycolipids fraction. This fraction was purified by reversed-phase C_{18} HPLC (CH_3CN/H_2O, 4:1) to give arabidopside F (**1**, 2.1 mg, 0.00035%) as colorless amorphous solid together with *sn*1-*O*-(12-oxophytodienoyl)-*sn*2-*O*-(hexadecatrienoyl)-monogalactosyl diglyceride (MGDG-O) (**2**, 2.4 mg, 0.00046%)[8], arabidopside A (**3**, 4.2 mg 0.00079%)[2], and arabidopside B (**4**, 1.3 mg, 0.00025%)[2].

Arabidopside F (**1**), $[\alpha]^{24}_D$ +20.0° (*c* 1.0, MeOH), showed the pseudomolecular ion peak at *m/z* 783 $(M+Na)^+$ in the ESIMS. HRESIMS analysis revealed the molecular formula to be $C_{43}H_{68}O_{11}$ [*m/z* 783.4659 $(M+Na)^+$, Δ+2.7 mmu]. The IR spectrum implied the presence of hydroxy (3423 cm^{-1}), ester carbonyl (1735 cm^{-1}), and unsaturated carbonyl (1685 and 1637 cm^{-1}) groups, while the UV absorption at 226 nm implied that **1** possessed an α, β-unsaturated ketone. The gross structure of arabidopside F (**1**) was deduced from detailed analysis of the ^{1}H and ^{13}C NMR data (Table 1) aided with 2D NMR experiments (^{1}H–^{1}H COSY, HMQC, and HMBC). The ^{13}C NMR data indicated that the molecule possessed one unsaturated carbonyl carbon, two ester carbonyl carbons, five disubstituted olefins, one acetal carbon, five oxymethines, three oxymethylenes, two methines, seventeen methylenes, and two methyl groups. The ^{1}H–^{1}H COSY connectivities of H-1 to H-3 and H-1' to H-6' indicated the presence of a glycerol and a sugar component. The sugar was assigned to be galactose by NOESY correlations of H-1'/H-3', H-1'/H-5', H-4'/H-3', and H-4'/H-5' and the ^{1}H–^{1}H coupling constants ($J_{1',2'}$ = 7.4 Hz, $J_{2',3'}$ =

Table 1. ^{1}H and ^{13}C NMR Data of Arabidopside F (**1**) in CD_3OD^a

Position	$^{1}H^{b}$		J(Hz)	$^{13}C^{b}$
1(a)	4.42	dd	12.0, 3.0	64.1
1(b)	4.23	dd	12.0, 6.4	
2	5.27	m		72.6
3(a)	3.98	dd	11.2, 5.7	69.6
3(b)	3.74	dd	11.2, 5.4	
1’	4.23	d	7.4	105.2
2’	3.51	m		73.3
3’	3.47	dd	10.0, 3.3	74.8
4’	3.83	d	3.3	71.9
5’	3.56	m		76.8
6’(a)	3.79	m		64.5
6’(b)	3.78	m		
1”				174.7
2”	2.31	m		34.8
3”	1.60	m		26.5
4”	1.23-1.42	m		28.2
5”	1.23-1.42	m		28.2
6”(a)	1.28	m		33.1
6”(b)	1.81	m		
7”	3.05	m		45.4
8”	7.92	dd	5.7, 2.7	170.4
9”	6.18	dd	5.7, 1.7	133.3
10”				214.1
11”	2.53	m		52.0
12”(a)	2.23	m		24.2
12”(b)	2.46	m		24.2
13”	5.27-5.46	m		134.7
14”				129.1
15”	2.17	m		21.7
16”	0.97	t	7.8	14.4
1’’’				174.7
2’’’	2.31	m		34.9
3’’’	1.60	m		26.5
4’’’-7’’’	1.23-1.42	m		28.2
				28.2
				30.2
				30.8
8’’’	2.09	m		26.0
9’’’	5.27-5.46	m		134.9
10’’’				126.1
11’’’	2.81	t	5.7	26.5
12’’’	5.27-5.46	m		131.1
13’’’				131.0
14’’’	2.81	t	5.7	26.0
15’’’	5.27-5.46	m		130.1
16’’’				132.8
17’’’	2.17	m		21.7
18’’’	0.97	t	7.8	14.6

[a]Data recorded on a 600 MHz spectrometer.
[b]in ppm

10.0 Hz, $J_{3',4'}$ = 3.3 Hz, and $J_{4',5'}$ = ~0 Hz). HMBC correlations of H-1’ to C-3 (δ_C 69.6) and H-3a and H -3b to C-1’ (δ_C 105.2) and the coupling constant ($J_{1',2'}$ = 7.4 Hz) of the anomeric proton (H-1’) at δ_H 4.23 revealed that arabidopside F (**1**) possessed a β-galactosylglycerol moiety.

The ^{1}H–^{1}H COSY connectivities of H-7’’ to H-9’’ and H-7’’ to H-11’’ and HMBC correlations of H-9’’ (δ_H 6.18) to C-7’’ (δ_C 45.4), C-10’’ (δ_C 214.1), and C-11’’ (δ_C 52.0) indicated the presence of one

cyclopentenone moiety. The ^1H–^1H COSY connectivities of H-11'' to H-16'' and HMBC correlations of Ha-12'' (δ_H 2.23) and Hb-12'' (δ_H 2.46) to C-13'' (δ_C 134.7) and C-14'' (δ_C 129.1) revealed that 2-pentene groups connected to C-11''. *Z*-Geometry of the disubstituted double bond at C-13''-C-14'' was deduced from the carbon chemical shifts of allylic carbons (C-12'', δ_C 24.2; C-15'', δ_C 21.7).[9]

These data and proton and carbon resonances indicated that arabidopside F (**1**) possessed two lipids containing a cyclopentenone. The one lipid was presumed to be *cis*-dinor-oxophytodienoic acid moiety judging from spectral data of *cis*-dinor-oxophytodienoic acid (dinor-OPDA).[10] An HCl/MeOH treatment of arabidopside F (**1**) afforded β-galactosylglycerol and two methyl esters of fatty acids, which were identified as methyl linolenate and methyl dinor-oxophytodienoate by the GC-MS analysis.[11] This result and the ^{13}C NMR data indicated that the another lipid was presumed to be linolenic acid. HMBC correlations of Ha-1 and Hb-1 to ester carbonyl carbon (δ_C 174.7) and chemical shifts (δ_H 5.27; δ_C 72.6) of C-2 indicated that the two fatty acid connected to C-1 and C-2. In order to define the locations of these lipids in the β-galactosylglycerol moiety of arabidopside F (**1**), we applied enzymatic hydrolysis. The lipase (from *Candida rugosa*, Sigma)-catalyzed hydrolysis of **1** afforded the mixture of a fatty acid and 1-*O*-deacylarabidopside F. After CH_2N_2 treatment of the reaction mixture, the residue was partitioned with hexane and 90% MeOH. Hexane soluble material was identical with methyl linolenate (*m/z* 292) by GC-MS analysis, while 90% MeOH soluble material was identified as *sn*2-*O*-(dinor-oxophytodienoyl)-monogalactosyl monoglyceride [ESIMS *m/z* 501 $(M+H)^+$] by LC-MS analysis. Therefore, arabidopside F (**1**) was assigned to be *sn*1-*O*-(linolenoyl)-*sn*2-*O*-(dinor-oxophytodienoyl) monogalactosyl diglyceride.

Arabidopside F (**1**) is a rare oxylipin containing dinor-OPDA, although MGDG-O (**2**), a chloroplast membrane oxylipin containing esterified OPDA, has been recently isolated from *A. thaliana*.[8] The OPDA and dinor-OPDA, which have been isolated from leaves of *A. thaliana* on wounding, suggested that they play role in wound signaling and these compounds are precursors of jasmonic acid. It may therefore be noted that the OPDA and dinor-OPDA in *A. thaliana* are present as arabidopsides A (**3**), B (**4**), F (**1**), and MGDG-O (**2**) in chloroplast membranes. The OPDA and dinor-OPDA can be released from chloroplast membranes enzymatically and this could account for the rapid transient increase in free OPDA, dinor-OPDA, and JA on the bioactivity such as wound signaling.[13]

As JA showed inhibitory effects on root growth of various plants,[3, 4] the effects of arabidopsides F (**1**), A (**3**), B (**4**), and MGDG-O (**2**) were also examined by the application to cress (*Lepidium sativum* L.) seeds. Ten seeds of cress were placed on a filter paper moistened with test solution and kept for 40 h at 24 ℃ in the dark, after which the lengths of their roots were measured. As shown in Figure 1, arabidopsides F (**1**), B (**4**), and MGDG-O (**2**) inhibited 20% of the growth of cress roots at 5×10^{-5} mol/L, while arabidopside A (**3**) inhibited 33% of the root growth at 5×10^{-5} mol/L.

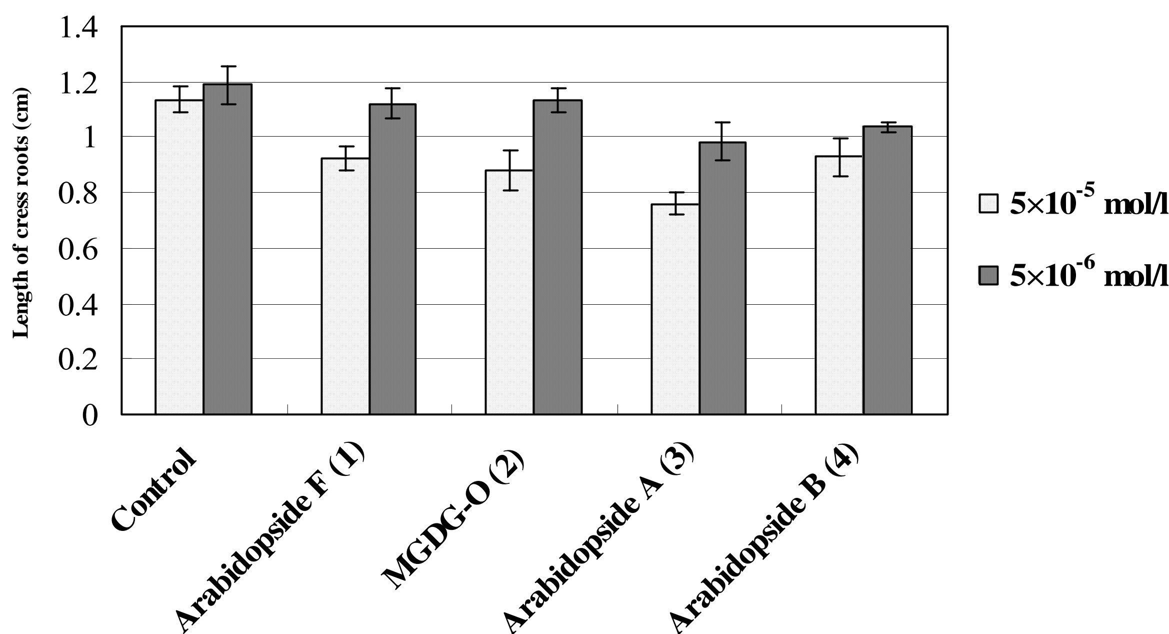


Figure 1. Growth inhibitory effects of arabidopside F (**1**)

EXPERIMENTAL

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 and δ_C 49.8 were used as internal references for NMR spectra. ESIMS were recorded on a micromass Q-Tof-2 mass spectrometer. GC-MS analysis were measured and recorded on a MS-6890 gas chromatograph and JMS-600H high resolution mass spectrometer.

Plant Material. The seeds of *Arabidopsis thaliana* ecotype Shokei (Brassicaceae) were purchased from SASSC. The seeds were immersed in H_2O at 4℃ for 2 days before sowing on rock wool (Rock fiber, NITTOBO, Japan). They were then cultured under continuous light (24 h, ca. 3,800 lux) at 24 °C until forming flower bud.

Extraction and Isolation. Aerial parts of *A. thaliana* (530 g) were extracted with MeOH (7 L), and evaporated to dryness *in vacuo* at 30 °C. The MeOH extract (14.7 g) was then partitioned between EtOAc (900 mL × 3) and H_2O (900 mL). The EtOAc-soluble portion (2.94 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 (4:1) was applied to a C_{18} Sep-Pak cartridge (CH_3CN/H_2O, 3:1, Waters) and the fraction (82.0 mg) containing oxylipins was further separated by reversed-phase HPLC [Deverosil ODS HG-5 (Nomura Chemical, ϕ 1.0×25 cm), flow rate 2.8 mL/min; solvent CH_3CN/H_2O (3:1); detection UV (222 nm)] to give arabidopside F (**1**, 2.1 mg, t_R 42.4 min) together with MGDG-O (**2**, 2.4 mg, t_R 38.8 min), arabidopsides A

(**3**, 4.2 mg, t_R 11.7 min) and B (**4**, 1.3 mg, t_R 17.5 min).

Arabidopside F (1). Colorless amorphous solid; $[\alpha]_D^{24}$ +20.0° (*c* 1.0, MeOH); IR (KBr) ν_{max} 3423, 1735, 1685, and 1637 cm^{-1}; UV(MeOH) λ_{max} 226 (ε 19200) nm; ESIMS (pos.) *m/z* 783 $(M+Na)^+$, HRESIMS (pos.) *m/z* 783.4659 $(M+Na)^+$, calcd for $C_{43}H_{68}O_{11}Na$, 783.4632.

Methanolysis of Arabidopside F (1). After a solution of arabidopside F (**1**, 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 (1 mL×3) and 90% MeOH (1 mL) and the hexane-soluble materials were used for GC-MS analyses.[11]

Enzymatic Hydrolysis of Arabidopside F (1). A solution of arabidopside F (**1**, 1.0 mg) and lipase from *Candida rugosa* (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.[12] The reaction was quenched with AcOH (0.1 mL), and then EtOH (2 mL) was added to the reaction mixture and the solvent was removed under reduced pressure. The residue was treated with CH_2N_2 (3 mL) and then was partitioned between hexane (1 mL×3) and 90% MeOH (1 mL). The hexane-soluble and 90% MeOH-soluble materials were used for GC-MS[11] and LC-MS analyses, respectively.

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% TritonX-100 (v/v) in a 3.3 cm Petri dish and kept for 40 h at 24℃ in the dark, after which the lengths of their roots were measured. Seedlings cultured on the solution containing 0.01% TritonX-100 was used as controls. Data are represented as mean values with standard errors of three experiments.

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