Pergamon

SCIENCE DIRECT®

Tetrahedron Letters 44 (2003) 5553–5556

TETRAHEDRON LETTERS

Arabidopsides A and B, two new oxylipins from *Arabidopsis thaliana*

Yosuke Hisamatsu,[a] Nobuharu Goto,[b] Koji Hasegawa[c] and Hideyuki Shigemori[c,*]

[a]*Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba 305-8572, Japan*
[b]*Department of Biology, Miyagi University of Education, Sendai 980-0845, Japan*
[c]*Institute of Applied Biochemistry, University of Tsukuba, Tsukuba 305-8572, Japan*

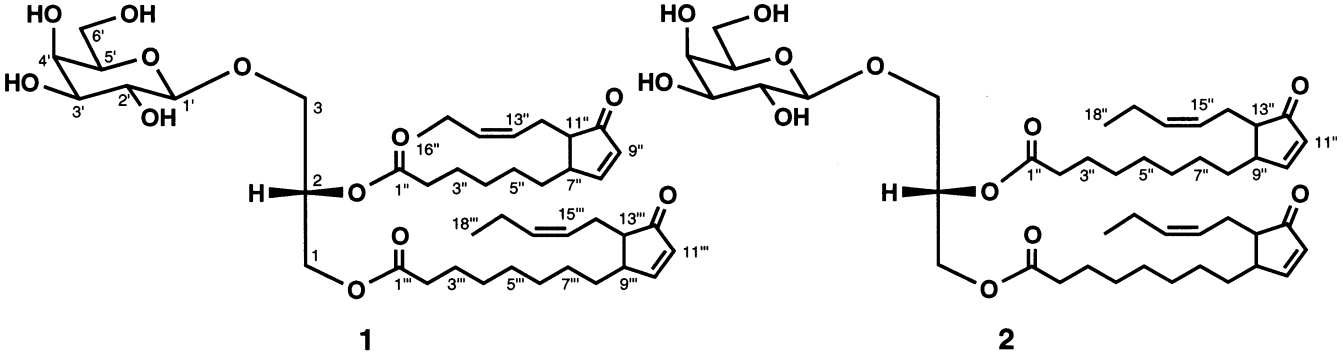
Received 20 March 2003; revised 28 April 2003; accepted 2 May 2003

Abstract—Two new oxylipins, arabidopsides A (**1**) and B (**2**), were isolated from the aerial parts of *Arabidopsis thaliana*, and their structures and absolute stereochemistries were elucidated by spectroscopic data and chemical means. Arabidopsides A (**1**) and B (**2**) were rare monogalactosyl diacylglycerides containing 12-oxophytodienoic acid and/or dinor-oxophytodienoic acid. © 2003 Elsevier Science Ltd. All rights reserved.

More than 10000 papers have been published on various aspects of the biology, chemistry, and genetics of *Arabidopsis thaliana* in the last three decades.[1] In our search for bioactive substances from *A. thaliana*, we isolated two new oxylipins, named arabidopsides A (**1**) and B (**2**), which were monogalactosyl diacylglycerides containing 12-oxophytodienoic acid and/or dinor-oxophytodienoic acid, from the aerial parts of this plant. In this paper we describe the isolation and structure elucidation of **1** and **2**.

The aerial parts (100 g) of *A. thaliana* ecotype col-0 (Brassicaceae) were extracted with MeOH. The MeOH extracts were partitioned with EtOAc and H_2O. The EtOAc-soluble portions were subjected to a silica gel column ($CHCl_3$/MeOH, 95:5) to afford a glycolipids fraction, which was purified by a reversed-phase C_{18}HPLC (CH_3CN/H_2O, 4:1) to give arabidopsides A (**1**, 4.0 mg, 0.004%) and B (**2**, 1.8 mg, 0.0018%) as colorless amorphous solids together with known monogalactosyl diacylglycerides.

The molecular formula, $C_{43}H_{66}O_{12}$, of arabidopside A (**1**),[2] $[\alpha]_D^{24}$ +56.8° (*c* 0.95, MeOH), was established by HRESIMS [*m*/*z* 792.4875 $(M+NH_4)^+$, Δ −2.3 mmu]. The IR spectrum implied the presence of hydroxy (3428 cm^{-1}), ester carbonyl (1738 cm^{-1}), and unsaturated carbonyl (1710 and 1630 cm^{-1}) groups. The gross structure of **1** was deduced from detailed analysis of the ^1H and ^{13}C NMR data (Table 1) aided with 2D NMR



1 **2**

Keywords: 12-oxophytodienoic acid; oxylipins; plants; natural products; *Arabidopsis thaliana*.
* Corresponding author. Fax: +81-29-853-4603; e-mail: hshige@agbi.tsukuba.ac.jp

0040-4039/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0040-4039(03)01148-1

Table 1. ^{1}H and ^{13}C NMR data of arabidopsides A (**1**) and B (**2**) in CD_3OD[a]

	1				**2**		
Position	^{1}H[b]	*J* (Hz)	^{13}C[b]	Position	^{1}H[b]	*J* (Hz)	^{13}C[b]
1(a)	4.48 dd	12.0, 3.1	64.78	1(a)	4.48 dd	12.2, 3.0	64.80
1(b)	4.25 dd	12.0, 6.5		1(b)	4.26 dd	12.2, 6.6	
2	5.30 m		72.67	2	5.30 m		72.64
3(a)	4.03 dd	11.0, 5.5	69.53	3(a)	4.03 dd	11.2, 5.6	69.54
3(b)	3.78 dd	11.0, 5.7		3(b)	3.76 dd	11.2, 6.1	
1′	4.27 d	7.6	106.16	1′	4.27 d	7.6	106.16
2′	3.53 m		73.19	2′	3.55 m		73.19
3′	3.51 dd	9.7, 3.3	75.69	3′	3.50 dd	9.6, 3.2	75.69
4′	3.87 d	3.3	71.03	4′	3.87 d	3.2	71.04
5′	3.53 m		77.62	5′	3.55 m		77.62
6′(a)	3.80 dd	11.4, 7.0	63.29	6′(a)	3.80 dd	11.4, 7.7	63.29
6′(b)	3.76 dd	11.4, 5.3		6′(b)	3.76 dd	11.4, 5.2	
1″,1‴			175.81	1″,1‴			175.56
			175.44				
2″,2‴	2.38 m		35.78	2″,2‴	2.37 m		35.88
			35.69				35.70
3″,3‴	1.66 m		26.76	3″,3‴	1.64 m		26.76
			26.62				
4″,4‴	1.38 m		31.08	4″,4‴	1.35 m		31.55
			31.03				
5″,5‴	1.38 m		29.40	5″,5‴	1.35 m		29.43
			29.17				
6‴	1.38 m		31.53	6″,6‴	1.35 m		32.63
7‴	1.38 m		30.88	7″,7‴	1.35 m		31.04
6″(a),8‴(a)	1.81 m		32.61	8″(a),8‴(a)	1.81 m		33.88
6″(b),8‴(b)	1.26 m		32.50	8″(b),8‴(b)	1.21 m		
7″,9‴	3.10 m		46.61	9″,9‴	3.09 m		46.62
			46.55				
8″,10‴	7.960 dd	5.9, 1.9	171.03	10″,10‴	7.93 dd	5.7, 2.2	171.03
	7.968 dd	5.9, 1.9	170.88				
9″,11‴	6.209 dd	5.9, 1.9	133.73	11″,11‴	6.20 dd	5.7, 2.2	133.67
	6.210 dd	5.9, 1.9	133.66				
10″,12‴			214.37	12″,12‴			214.74
			214.28				
11″,13‴	2.56 m		51.79	13″,13‴	2.52 m		51.58
			51.79				
12″,14‴	2.47 m		25.76	14″,14‴	2.46 m		25.76
	2.21 m		25.76		2.21 m		
13″,15‴	5.45 m		129.09	15″,15‴	5.44 m		129.08
			129.03				
14″,16‴	5.45 m		134.63	16″,16‴	5.44 m		134.58
			134.57				
15″,17‴	2.12 m		22.57	17″,17‴	2.11 m		22.57
			22.57				
16″,18‴	1.02 t	7.5	15.47	18″,18‴	1.02 t	7.5	15.22
			15.23				

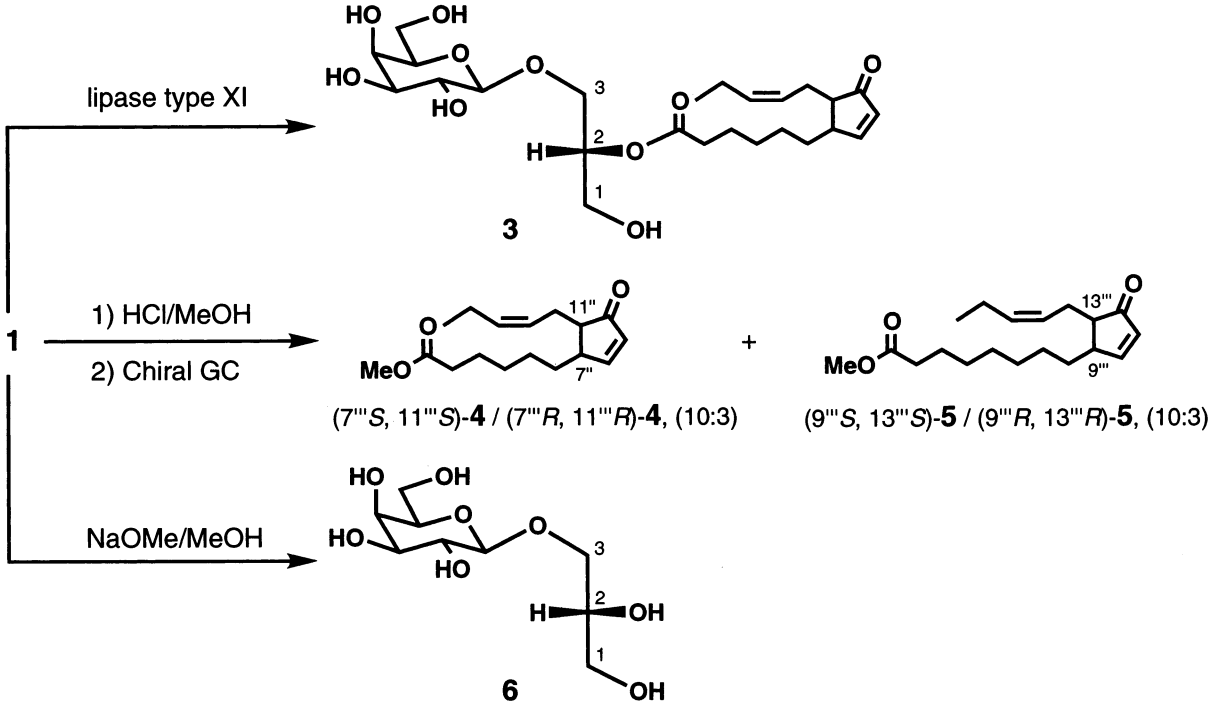
[a] Data recorded on a 500 MHz spectrometer with reference to the solvent signals (δ_H 3.35, δ_C 49.8).
[b] In ppm.

experiments (^{1}H–^{1}H COSY, HMQC, and HMBC). The ^{13}C NMR data indicated that the molecule possessed two unsaturated carbonyl carbons, two ester carbonyl carbons, four disubstituted olefins, one acetal carbon, five oxymethines, three oxymethylenes, four methines, sixteen methylenes, and two methyl groups. The ^{1}H–^{1}H COSY connectivities of C-1 to C-3 and C-1′ to C-6′ indicated the presence of a glycerol and a sugar component. The sugar was assigned to be galactose by NOESY correlations of H-1′ to H-3′ and H-5′ and H-4′ to H-3′ and H-5′ and the ^{1}H–^{1}H coupling constants ($J_{1',2'}=7.6$ Hz, $J_{2',3'}=9.7$ Hz, $J_{3',4'}=3.3$ Hz, and $J_{4',5'}=\sim 0$ Hz). HMBC correlations of H-1′ to C-3 (δ_C 69.53) and H-3a and H-3b to C-1′ (δ_C 106.16) and the coupling constant ($J_{1',2'}=7.6$ Hz) of the anomeric proton (H-1′) at δ_H 4.27 revealed that **1** possessed a β-galactosylglycerol moiety. The ^{1}H–^{1}H COSY connectivities of C-7″ (or C-9‴) to C-9″(or C-11‴) and C-7″ (or C-9‴) to C-11″ (or C-13‴) and HMBC correlations of H-9″ (or H-11‴) (δ_H 6.210 or 6.209) to C-7″ (or C-9‴) (δ_C 46.61 or 46.55), C-10″ (or C-12‴) (δ_C 214.37 or 214.28), and C-11″ (or C-13‴) (δ_C 51.79) indicated the presence

of two cyclopentenone moieties. The ^1H–^1H COSY connectivities of C-11″ (or C-13‴) to C-16″ (or C-18‴) and HMBC correlations of Ha-12″ (or Ha-14‴) (δ_H 2.47) and Hb-12″ (or Hb-14‴) (δ_H 2.21) to C-13″ (or C-15‴) (δ_C 129.09 or 129.03) and C-14″ (or C-16‴) (δ_C 134.63 or 134.57) revealed that 2-pentene groups connected to C-11″ and C-13‴. *Z*-Geometries of two disubstituted double bonds at C-13″–C-14″ and C-15‴–C-16‴ were deduced from the carbon chemical shifts of allylic carbons (C-12″ or C-14‴, δ_C 25.76; C-15″ or C-17‴, δ_C 22.57).[3] These data and proton and carbon resonances indicated that **1** possessed two lipids containing cyclopentenone. The two lipids were presumed to be *cis*-12-oxophytodienoic acid and *cis*-dinor-oxophytodienoic acid moieties judging from spectral data of *cis*-12-oxophytodienoic acid (OPDA)[4] and *cis*-dinor-oxophytodienoic acid (dinor-OPDA).[5] HMBC correlations of Ha-1 and Hb-1 to ester carbonyl carbon (δ_C 175.81 or 175.44) and chemical shifts (δ_H 5.30; δ_C 72.67) of C-2 indicated that the OPDA and dinor-OPDA connected to C-1 and C-2. In order to define the locations of these lipids in the β-galactosylglycerol moiety of **1**, we applied enzymatic hydrolysis. The lipase type XI (Sigma)-catalyzed hydrolysis of **1** afforded 1-*O*-deacylarabidopside A (**3**) [ESIMS *m*/*z* 523 (M+Na)$^+$] (Scheme 1).[6] Thus, arabidopside A (**1**) was assigned to be *sn*1-*O*-(12-oxophytodienoyl)-*sn*2-*O*-(dinor-oxophytodienoyl)-monogalactosyl diglyceride. The chiral GC-analyses (γ-DEX™ 120 Capillary Column, SUPELCO) of methanolysates of **1** by treatment with HCl/MeOH detected the mixture (10:3) of (7″*S*,11″*S*)-**4** and (7″*R*,11″*R*)-**4** and the mixture (10:3) of (9‴*S*,13‴*S*)-**5** and (9‴*R*,13‴*R*)-**5** (Scheme 1).[7] The absolute configuration at C-2 in the β-galactosylglycerol (**6**), which was derived from **1** with NaOMe,[8] was presumed to be *R*, from the basis of a comparison of the optical rotation of **6** ($[\alpha]_D$ −7°) with the reported values ($[\alpha]_D$ −7° for C-2 *R* and $[\alpha]_D$ +2° for C-2 *S*) (Scheme 1).[9] Therefore, the absolute configuration at C-2 of **1** has been assigned as *S*, while the dinor-OPDA and OPDA of **1** have been enantiomeric mixture (10:3) of (7″*S*,11″*S*), (9‴*S*,13‴*S*)- form and (7″*R*,11″*R*), (9‴*R*,13‴*R*)-form.

Arabidopside B (**2**),[10] $[\alpha]_D^{26}$ +61.1° (*c* 0.48, MeOH), showed the pseudomolecular ion peak at *m*/*z* 820 (M+NH$_4$)$^+$ in the ESIMS. HRESIMS analysis revealed the molecular formula to be $C_{45}H_{70}O_{12}$ [*m*/*z* 820.5206 (M+NH$_4$)$^+$, Δ −0.5 mmu], indicating that **2** assumed to be an ethylene homolog of **1**. The ^1H and ^{13}C NMR spectra of **2** were similar to those of arabidopside A (**1**), except for the signals due to the methylene parts. Treatment of **2** with HCl/MeOH afforded 2 mol of OPDA. Therefore, arabidopside B (**2**) was assigned to be *sn*1, *sn*2-di-*O*-(12-oxophytodienoyl)-monogalactosyl diglyceride. The chiral GC-analyses of methanolysates of **2** revealed the mixture (10:3) of enantiomers of (9″*S*,13″*S*), (9‴*S*,13‴*S*)-form and (9″*R*,13″*R*), (9‴*R*,13‴*R*)-form and the absolute configuration of C-2 was assigned as *S* judging from optical rotation of **6** ($[\alpha]_D$ −10°), which was obtained from methanolysis of **2** with NaOMe.

Arabidopsides A (**1**) and B (**2**) are rare oxylipins containing OPDA and dinor-OPDA, although *sn*1-*O*-(12-oxophytodienoyl)-*sn*2-*O*-(hexadecatrienoyl)-monogalactosyl diglyceride, a chloroplast membrane oxylipin containing esterified OPDA, has been recently isolated from *A. thaliana*.[11] The OPDA and dinor-OPDA, which have been isolated from leaves of *A. thaliana* on



Scheme 1.

wounding, suggested roles 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 (**1**) and B (**2**), and *sn*1-*O*-(12-oxophytodienoyl)-*sn*2-*O*-(hexadecatrienoyl)-monogalactosyl diglyceride 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 jasmonic acid on the bioactivity such as wound signaling.[12]

Acknowledgements

We thank Dr. S. Matsuyama (University of Tsukuba) for chiral GC analyses and Banyu Pharmaceutical Co., Ltd. for measurement of ESIMS. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan.

References

1. Veit, M.; Pauli, G. F. *J. Nat. Prod.* **1999**, *62*, 1301–1303.
2. Compound **1**: IR (KBr) ν_{max} 3428, 1738, 1710, 1630, 1170 and 1073 cm^{-1}; UV (MeOH) λ_{max} 222 (ε 11000) nm.
3. The allylic carbons of *cis*- and *trans*-double bonds in the aliphatic chains are reported to resonate approximately at 27 and 32 ppm, respectively: Gunstone, F. D.; Pollard, M. R.; Scrimgeour, C. M.; Vedanayagam, H. S. *Chem. Phys. Lipids* **1977**, *18*, 115–129.
4. Baertschi, S. W.; Ingram, C. D.; Harris, T. M.; Brash, A. R. *Biochemistry* **1988**, *27*, 18–24.
5. Weber, H.; Vick, B. A.; Farmer, E. E. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 10473–10478.
6. Enzymatic hydrolysis of **1**. A solution of **1** (1 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 was added to the reaction mixture. The solvent was removed under reduced pressure and the resulting residue was purified on a silica gel column ($CHCl_3$/MeOH, 7:1) to yield **3** (0.4 mg, 62%). Morimoto, T.; Nagatsu, A.; Murakami, N.; Sakakibara, J.; Tokuda, H.; Nishino, H.; Iwashima, A. *Phytochemistry* **1995**, *40*, 1433–1437.
7. Methanolysis of **1** and the chiral GC analyses of the methanolysates. After a solution of **1** (0.2 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 with hexane and 90% MeOH and the hexane-soluble materials were used for chiral GC analyses. 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 OPDA and dinor-OPDA were determined by the retention time (min). The *cis* enantiomers, (7″*S*,11″*S*)-**4** (238 min) and (7″*R*,11″*R*)-**4** (238 min) and (9‴*S*,13‴*S*)-**5** (397 min) and (9‴*R*,13‴*R*)-**5** (397 min) were inseparable, respectively, while the *trans* enantiomers, (7″*S*,11″*R*)-**4** (157 min) and (7″*R*,11″*S*)-**4** (158 min) (10:3) and (9‴*S*,13‴*R*)-**5** (359 min) and (9‴*R*,13‴*S*)-**5** (363 min) (10:3), which were produced from *cis* forms by enolization. Laudert, D.; Hennig, P.; Stelmach, B. A.; Müller, A.; Andert, L.; Weiler, E. W. *Anal. Biochem.* **1997**, *246*, 211–217.
8. Methanolysis of **1** with NaOMe. A solution of **1** (0.9 mg) and NaOMe (10 equiv.) in anhyd. MeOH (0.1 mL) was stirred at room temperature for 1 h. The reaction mixture was partitioned between hexane and water and the water-soluble materials were purified by C_{18} column (MeOH/H_2O, 1:1) to give the β-galactosylglycerol (**6**, 0.27 mg, 92%).
9. Son, B. W.; Cho, Y. J.; Choi, J. S.; Lee, W. K.; Kim, D.-S.; Choi, H. D.; Choi, J. S.; Jung, J. H.; Im, K. S.; Choi, W. C. *Nat. Prod. Lett.* **2001**, *15*, 299–306.
10. Compound **2**: colorless amorphous solid; IR (KBr) ν_{max} 3430, 1738, 1710(sh), 1631, 1166, and 1069 cm^{-1}; UV (MeOH) λ_{max} 225 (ε 13200) nm.
11. Stelmach, B. A.; Müller, A.; Hennig, P.; Gebhardt, S.; Schubert-Zsilavecz, M.; Weiler, E. W. *J. Biol. Chem.* **2001**, *276*, 12832–12838.
12. Turner, J. G.; Ellis, C.; Devoto, A. *The Plant Cell* **2002**, S153–S164.