Phytochemistry 69 (2008) 2793–2798

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Detection of divinyl ether synthase in Lily-of-the-Valley (*Convallaria majalis*) roots

Anna V. Ogorodnikova, Larisa R. Latypova, Fahima K. Mukhitova, Lucia S. Mukhtarova, Alexander N. Grechkin *

Kazan Institute of Biochemistry and Biophysics, Russian Academy of Sciences, Lobachevsky Street 2/31, P.O. Box 30, 420111 Kazan, Russia

ARTICLE INFO

Article history:
Received 23 August 2008
Received in revised form 10 September 2008
Available online 24 October 2008

Keywords:
Divinyl ether synthase
Oxylipins
Divinyl ethers
Etheroleic acid
Biosynthesis
Lily-of-the-Valley (*Convallaria majalis* L.)

ABSTRACT

Incubations of linoleic acid with cell-free preparations from Lily-of-the-Valley (*Convallaria majalis* L., Ruscaceae) roots revealed the presence of 13-lipoxygenase and divinyl ether synthase (DES) activities. Exogenous linoleic acid was metabolized predominantly into (9*Z*,11*E*,1′*E*)-12-(1′-hexenyloxy)-9,11-dodecadienoic (etheroleic) acid. Its identification was confirmed by the data of ultraviolet spectroscopy, mass spectra, ^{1}H NMR, COSY, catalytic hydrogenation. The isomeric divinyl ether (8*E*,1′*E*,3′*Z*)-12-(1′,3′-nonadienyloxy)-8-nonenoic (colneleic) acid was detected as a minor product. Incubations with linoleic acid hydroperoxides revealed that 13-hydroperoxide was a preferential substrate, while the 9-hydroperoxide was utilized with lesser efficiency.

1. Introduction

Plant oxylipins constitute a family of bioregulators involved into the cell signalling and defence (Blée, 1998; Grechkin, 1998). These products are biosynthesized through the oxidative metabolism of polyenoic fatty acids controlled by lipoxygenases. There are a number of known routes of the plant lipoxygenase cascade. The primary lipoxygenase products, fatty acid hydroperoxides, are further metabolized by a few major enzymes. These enzymes belong to CYP74 family of P450 macrofamily. There are three main types of CYP74 enzymes: allene oxide synthase (AOS), hydroperoxide lyase (HPL) and divinyl ether synthase (DES) (Blée, 1998; Grechkin, 1998, 2002; Stumpe and Feussner, 2006). DES is less characterized than AOS and HPL. As shown recently, divinyl ethers (DES products), possess potent antimicrobial activity (Granér et al., 2003; Cowley and Walters, 2005). DES gene expression in plants is initiated by pathogen attack (Weber et al., 1999; Göbel et al., 2001; Stumpe et al., 2001; Fammartino et al., 2007). Thus, DES and divinyl ethers are involved into the plant defence towards pathogens.

Although DES was found yet in a limited number of plant species, the known species possessing DES activity are phylogenetically distant. These are species of brown (Proteau and Gerwick, 1993); and red (Jiang and Gerwick, 1997) algae, monocots (garlic) (Grechkin et al., 1995; Grechkin and Hamberg, 1996, Grechkin et al., 1997; Stumpe et al. 2008) and dicots, including several species of Ranunculaceae (Hamberg, 1998, 2002, 2004, 2005) and Solanaceae, including potato (Galliard and Phillips, 1972; Galliard et al., 1973; Galliard and Mathew, 1975; Stumpe et al., 2001), tomato (Itoh and Howe, 2001) and tobacco (Fammartino et al., 2007). Genes of tomato (Itoh and Howe, 2001), tobacco (Fammartino et al., 2007) and garlic (Stumpe et al., 2008) DESs have been cloned. The present state of knowledge on CYP74 family and especially DESs remains largely incomplete. The present paper reports the detection of a novel DES in the roots of Lily-of-the-Valley (*Convallaria majalis* L., Ruscaceae). Lily-of-the-Valley presents a second example (along with garlic) of monocot species possessing DES.

2. Results

2.1. Detection of divinyl ethers

For the preliminary characterization of the in vitro metabolism, the 15,000 *g* supernatant of *C. majalis* root homogenate was incubated with linoleic acid. Analysis of products (as trimethylsilylated methyl esters) by GC–MS (Fig. 1a) revealed formation of one predominant oxylipin **1a**. Its mass spectrum (Table 1) and GC

Abbreviations: DES, divinyl ether synthase; 13-HPOD, (9*Z*,11*E*,13*S*)-13-hydroperoxy-9,11-octadecadienoic acid; 9-HPOD, (9*S*,10*E*,12*Z*)-9-hydroperoxy-10,12-octadecadienoic acid; AOS, allene oxide synthase; HPL, hydroperoxide lyase; 12-oxo-PEA, 12-oxo-10-phytoenoic acid; RP-HPLC, reversed phase HPLC; NP-HPLC, normal phase HPLC.

* Corresponding author. Tel.: +7 843 292 75 35; fax: +7 843 292 73 47.
E-mail address: grechkin@mail.knc.ru (A.N. Grechkin).

0031-9422/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2008.09.011

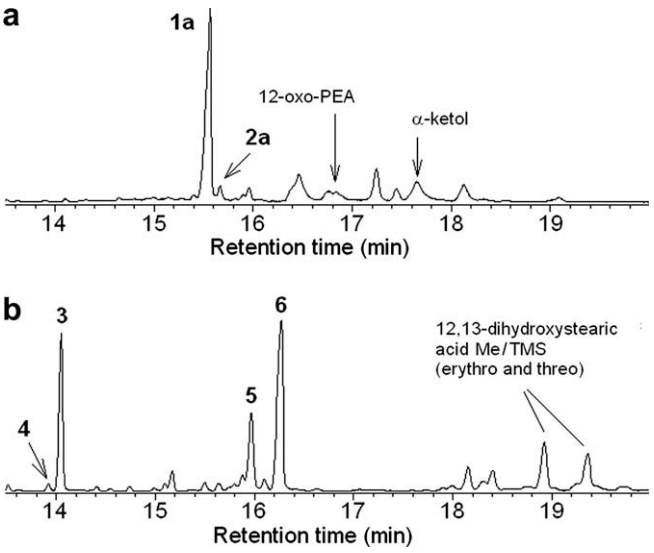


Fig. 1. Products of linoleic acid incubation with 15,000 *g* supernatant of homogenate of Lily-of-the-Valley roots. The total ion GC–MS chromatograms of products as Me esters (a) or $NaBH_4$ reduced hydrogenated products as Me esters TMSi derivatives.

retention time matched those for divinyl ether etheroleic acid, (9*Z*,11*E*,1′*E*)-12-(1′-hexenyloxy)-9,11-dodecadienoic acid methyl ester. Another product **2a** possessed the same molecular mass (*m/z* 308, Table 1), but distinctive fragmentation patterns. Its mass spectrum corresponds to that for isomeric divinyl ether colneleic acid, (8*E*,1′*E*,3′*E*)-12-(1′,3′-nonadienyloxy)-8-nonenoic acid methyl ester.

GC–MS analysis was repeated after the treatment of the same incubation products with (i) sodium borohydride, (ii) diazomethane, (iii) catalytic hydrogenation, (iv) trimethysilylation. GC—The data of GC–MS analysis of the resulting saturated Me esters TMSi derivatives are presented in Fig. 1b. Inter alia, two unpolar products **3** (major one) and **4** (minor one) were detected. Mass spectrum of compound **3** possessed a molecular ion at *m/z* 314 and characteristic fragmentation patterns (Table 1) that enable one to identify compound **3** unequivocally as a saturated ether 13-oxa-nonadecanoic acid. This is in full agreement with identification of major metabolite **1a** as etheroleic acid.

The minor unpolar hydrogenation product **4** possessed characteristic mass spectral patterns (Table 1), which enabled us to identify it as 10-oxa-nonadecanoic acid. This saturated ether is evidently the product of hydrogenation of colneleic acid. These data confirm the identification of compound **2a** as colneleic acid.

2.2. Specificity of linoleic acid metabolism via the lipoxygenase-divinyl ether synthase pathway

Analyses of fully reduced and hydrogenated product derivatives (Fig. 1b) allowed one to detect compounds **5** and **6**. Their mass spectral data (Table 1) enable us to identify them unequivocally as 9-hydroxystearic (Me/TMSi) and 13-hydroxystearic (Me/TMSi), respectively. Their relative abundance is about 1:2 (as seen from total ion current chromatogram, Fig. 1b). This data demonstrate that both 13- and 9-lipoxygenase activities are present in *C. majalis* roots. Although acid is turned into both 13-HPOD and 9-HPOD, etheroleic acid formation predominates over colneleic acid biosynthesis (Fig. 1). Thus, major route of the lipoxygenase pathway in *C. majalis* roots is under control of 13-lipoxygenase and DES.

Both 13-HPOD (Fig. 2a) and 9-HPOD (Fig. 2b) where converted to products **1a** and **2a** (etheroleic and colneleic acids, respectively).

Table 1
Mass spectral data for compounds **1a**, **2a**, **3**, **4**, **5** and **6**

Ion attribution	Products					
	1a	**2a**	**3**	**4**	**5**	**6**
M^+	306 (37)	308 (13)	314 (1)	314 (1)		
M^+–H					385 (0.3)	385 (0.2)
M^+–CH_3					371 (2)	371 (1)
M^+–CH_3O	277 (3)	277 (1)	283 (3)	283 (2)		
M^+–CH_3OH					356 (1)	356 (1)
371–CH_3OH					339 (8)	339 (9)
M^+–$(CH_2)_3CH_3$	251 (5)	251 (5)				
M^+–$(CH_2)_4CH_3$			243 (4)			315 (53)
M^+–TMSiOCH$(CH_2)_4CH_3$ +TMSi						286 (14)
M^+–$(CH_2)_5CH_3$			229 (33)			
M^+–$O(CH_2)_5CH_3$ +H			214 (27)			
M^+–$(CH_2)_7CH_3$				201 (6)		
229–MeOH			197 (75)			
M^+–$(CH_2)_8CH_3$				187 (26)	259 (100)	
M^+–$O(CH_2)_5CH_3$–MeOH			181 (17)			
M^+–$O(CH_2)_8CH_3$ +H				172 (25)		
187–MeOH				155 (79)		
M^+–$(CH_2)_7$COOMe					229 (80)	
M^+–$(CH_2)_{11}$COOMe						173 (100)
M^+–$CH_3(CH_2)_3CH$=CHO	209 (3)					
209–CH_3OH	177 (24)					
M^+–$(CH_2)_6$COOMe	165 (26)	165 (6)				
M^+–$(CH_2)_8$COOMe				143 (24)		
Not assigned	159 (23)		163 (23)	138 (70)	155 (31)	
Not assigned	149 (16)		143 (50)			
M^+–$CH_3(CH_2)_4CH$=CH–CH=CHO–CH_3OH		137 (14)				
Not assigned	135 (39)		129 (16)		109 (18)	159 (8)
Not assigned	95 (68)	95 (40)	97 (54)	97 (57)		
Not assigned	81 (97)	81 (81)	87 (100)	87 (84)		
Not assigned	67 (100)	67 (100)	74 (96)	74 (88)		
CH_2=O^+SiMe_3					103 (15)	103 (14)
Not assigned					83 (38)	
$(CH_3)_2Si$=O^+H					75 (40)	75 (35)
$TMSi^+$					73 (95)	73 (68)

Unlike 9-HPOD (Fig. 2b), 13-HPOD (Fig. 2a) was fully transformed into a divinyl ether. Thus, the enzyme possessed the preference towards 13-HPOD.

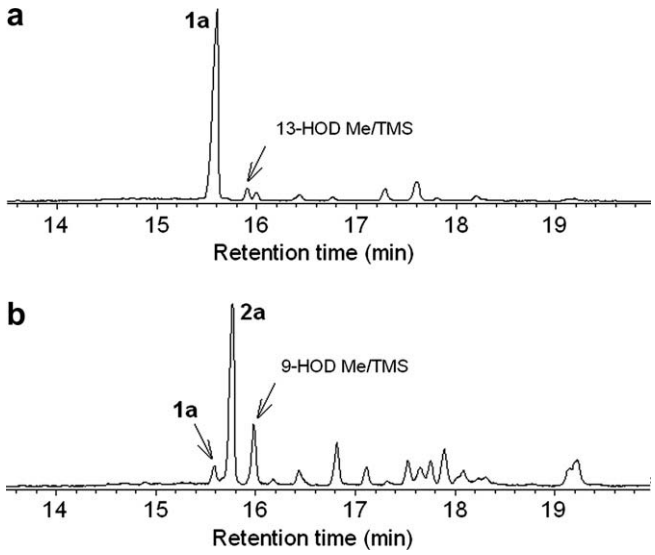


Fig. 2. Products of 13-HPOD (a) and 9-HPOD (b) incubations with 15,000 *g* supernatant of homogenate of Lily-of-the-Valley roots. The total ion GC–MS chromatograms of products as Me esters TMSi derivatives.

2.3. Purification of compound 1a and studies of its structure by ^{1}H NMR and 2D-COSY

For final structural confirmation and elucidation of its geometrical configuration, compound **1a** was purified and its ^{1}H NMR and COSY spectra were recorded. For preparation of compound **1**, the cell-free preparation from *C. majalis* roots was incubated with [1-^{14}C]13-HPOD for 10 min. Fig. 3 presents the RP-HPLC analysis of products (as methyl esters) with simultaneous radiodetection (Fig. 3a) and ultraviolet absorbance detection (Fig. 3b) by diode array detector. The major product **1a** (λ_{max} at 251 nm) was collected and then purified by NP-phase HPLC (see Scheme 1).

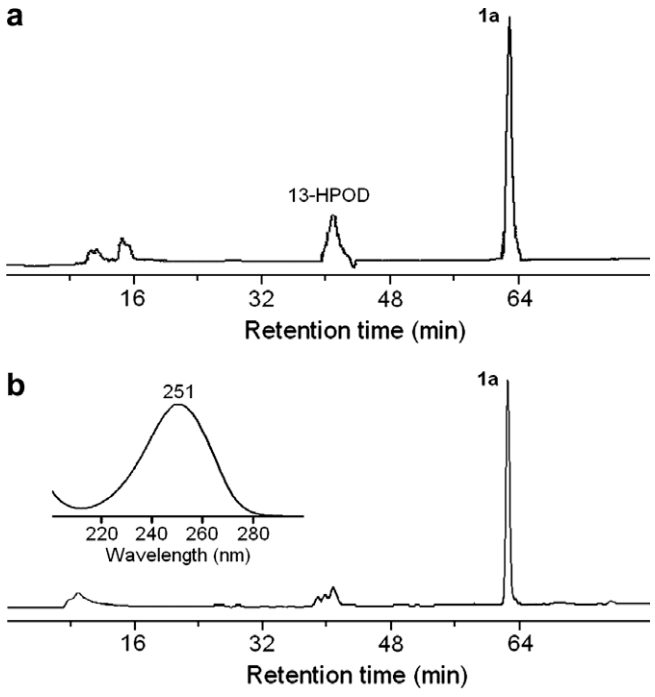
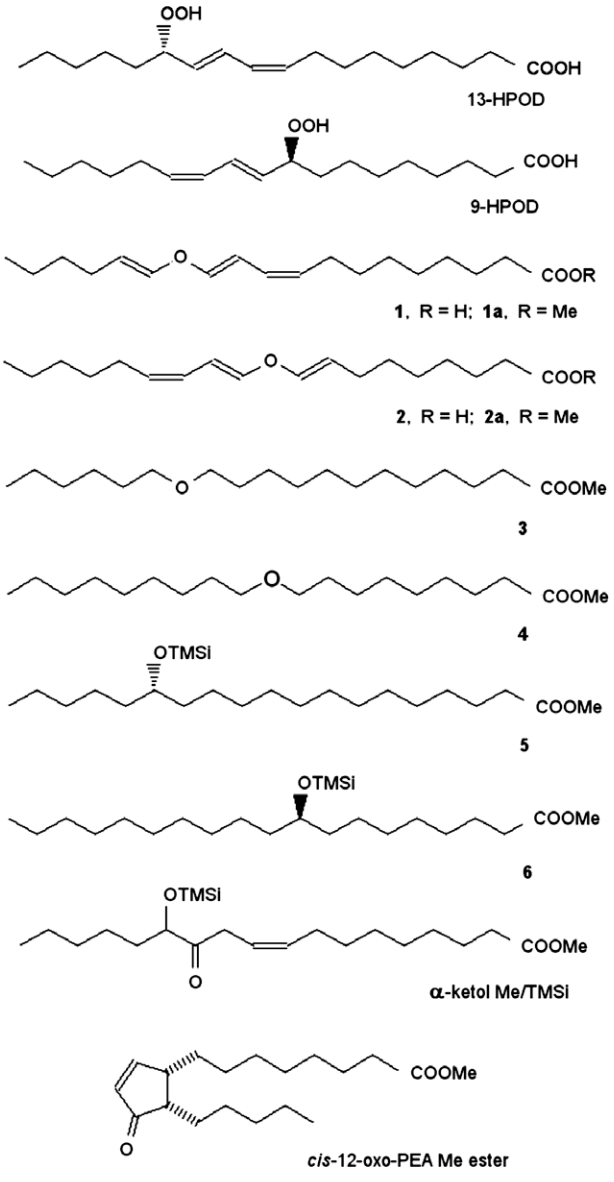


Fig. 3. Products (Me esters) of [1-^{14}C]13-HPOD incubation with enzyme from Lily-of-the-Valley roots obtained by the $(NH_4)_2SO_4$ precipitation as described under Materials and Methods. The RP-HPLC analysis with the online radiodetection (a) and the parallel diode array detection, represented by 250 nm chromatogram (b). Insert, the ultraviolet spectrum for compound **1a**.

The ^{1}H NMR (Table 2) and COSY (Fig. 4) data confirmed that compound **1a** has three double bonds. The COSY data (Fig. 4) enable one to confirm the assignment of all signals. The spin coupling constant values (Table 2) show that the double bonds have (9*Z*,11*E*,1′*E*) configuration. The obtained NMR data for compound **1a** fully correspond to those previously described for etheroleic acid methyl ester (Grechkin et al., 1995, 1997). Thus, the obtained data enable us to identify compound **1** as etheroleic acid, i.e. (9*Z*,11*E*,1′*E*)-12-(1′-hexenyloxy)-9,11-dodecadienoic acid.

2.4. Identification of allene oxide synthase products

The results described above demonstrated that Lily-of-the-Valley roots possess predominantly the 13-lipoxygenase – divinyl ether synthase pathway. At the same time, some minor metabolites were formed, which are worth to be mentioned.



Scheme 1.

Table 2
^{1}H NMR spectral data for etheroleic acid methyl ester (**1a**) (400 MHz, C^2H_3CN, 296 K)

Proton	Chemical shift, δ (ppm)	Multiplicity (number of protons)	Coupling constants (Hz)
H2	2.30	*t* (2)	7.5 (H3)
H3	1.62	*m* (2)	
H4	1.31	*m* (2)	
H5	1.31	*m* (2)	
H6	1.31	*m* (2)	
H7	1.37	*m* (2)	
H8	2.09	*ddt* (2)	7.3 (H7); 7.5 (H9); 1.3 (H10)
H9	5.28	*dt* (1)	10.6 (H10)
H10	5.85	*ddt* (1)	11.1 (H11)
H11	5.98	*ddd* (1)	11.9 (H12); 0.9 (H9)
H12	6.51	*d* (1)	
H1′	6.26	*dt* (1)	12.2 (H2′); 1.3 (H3′)
H2′	5.15	*dt* (1)	7.5 (H3′)
H3′	1.96	*ddt* (2)	7.3 (H4′)
H4′	1.35	*m* (2)	
H5′	1.31	*m* (2)	
H6′	0.89	*t* (3)	7.0 (H5′)
H(OMe)	3.66	*s* (3)	

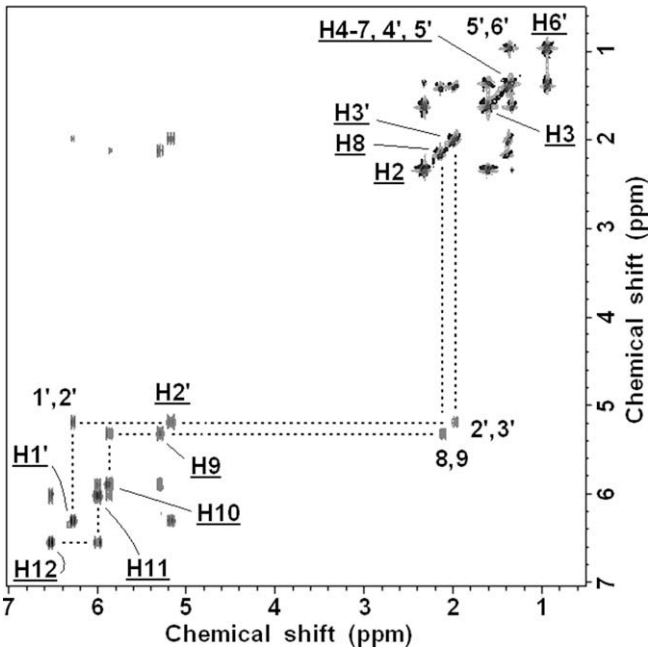


Fig. 4. The 2D-COSY plot for etheroleic acid methyl ester (**1a**) (400 MHz, C_2H_3CN, 296 K).

These are the products of allene oxide synthase (AOS) pathway, inter alia α-ketol (9*Z*)-12-oxo-13-hydroxy-9-octadecenoic acid (detected as methyl ester TMSi derivative) and the cyclopentenone 12-oxo-10-phytoenoic acid (12-oxo-PEA) methyl ester, as illustrated in Fig. 1. The spectral data for these products are not presented. The mass spectra fully correspond to that recently published for these compounds (Grechkin et al., 2007). After the sequential treatment of incubation products with sodium borohydride, diazomethane, catalytic hydrogenation and trimethylsilylation α-ketol turned into two (erythro and threo) saturated vicinal diols Me/TMSi derivatives (Fig. 1b). As well as DES, AOS utilizes 13-hydroperoxides as preferential substrates, while 9-HPOD was a poor substrate. Only a little amount of α-ketol (12*Z*)-9-hydroxy-10-oxo-12-octadecenoic acid Me/TMSi derivative was detectable after the incubation with 9-HPOD.

3. Discussion

The obtained results show that Lily-of-the-Valley (*C. majalis* L.) appends the limited number of plants possessing DES activity and containing divinyl ethers. DES was detected before in only one monocot species, namely garlic (*Allium sativum* L.) (Grechkin et al., 1995; Grechkin and Hamberg, 1996; Grechkin et al., 1997). Lily-of-the-Valley and garlic are not closely related species, belonging to different monocot families, Ruscaceae and Alliaceae, respectively. DESs of both species possess similar specificity. Both of them utilize preferentially 13-HPOD and produce (9*Z*,11*E*,1′*E*)-12-(1′-hexenyloxy)-9,11-dodecadienoic (etheroleic) acid.

Divinyl ethers are involved into plant defence against pathogens, being antimicrobial compounds (Weber et al., 1999; Göbel et al., 2001; Stumpe et al., 2001; Granér et al., 2003; Cowley and Walters, 2005; Fammartino et al., 2007). DES genes are constitutively expressed in the roots of tomato (Itoh and Howe, 2001) and tobacco (Fammartino et al., 2007), but DES activity is significantly increased upon the infection of tobacco (Fammartino et al., 2007). DES genes in the leaves and other organs (except roots) of Solanaceae plants are specifically expressed in response to pathogen attack (Weber et al., 1999; Fammartino et al., 2007). The results of present work show that Lily-of-the-Valley possesses different defence strategies: DES gene is expressed constitutively in Lily-of-the-Valley roots, as well as in garlic bulbs (Grechkin et al., 1995, 1997; Grechkin and Hamberg, 1996; Stumpe et al., 2008), in flax leaves (Chechetkin et al., 2008) and in Ranunculaceae plants (Hamberg, 1998, 2002, 2004, 2005).

DES genes or DES activities were detected yet in a limited number of plant species. At the same time, these are phylogenetically distant species, including the brown (Proteau and Gerwick, 1993;) and red (Jiang and Gerwick, 1997) algae, monocots (garlic) (Grechkin et al., 1995, 1997; Grechkin and Hamberg, 1996; Stumpe et al. 2008) and dicots, inter alia some species of Ranunculaceae (Hamberg, 1998, 2002, 2004, 2005) and Solanaceae, including potato (Galliard and Phillips, 1972; Galliard et al., 1973; Galliard and Mathew, 1975; Stumpe et al., 2001), tomato (Itoh and Howe, 2001) and tobacco (Fammartino et al., 2007). Lily-of-the-Valley enzyme is a second monocot species along with garlic possessing DES.

Lack of the detectable DES activity in some plant species does not necessarily indicate the absence of DES genes in their genomes. One can propose that DES is detected in lesser number of species as compared to AOS and HPL because DES genes of many plants are silent under the normal conditions. The current state of knowledge even about the sequenced genomes is not complete. For instance, the *Arabidopsis thaliana* Genome Database (Plant Genome Database) includes only two CYP74 genes (AOS and HPL), while at least few more CYP74 partly sequenced mRNA are registered in the *A. thaliana* Transcript Database and *A. thaliana* EST_Contig (Plant Genome Database). Nevertheless, the knowledge on DES occurrence is extending. This results of this paper as well as the previous one (Chechetkin et al., 2008) provide new examples of plant species possessing DES.

4. Experimental

4.1. Materials

Unlabelled linoleic acid and lipoxygenase type V were purchased from Sigma. Sodium borohydride as well as the silylating reagents were purchased from Fluka (Buchs, Switzerland). Lily-of-the-Valley (*C. majalis*) roots were collected at gardens nearby Kazan.

4.2. Substrate preparations

(9*Z*,11*E*,13*S*)-13-Hydroperoxy-9,11-octadecadienoic acid (13-HPOD) was prepared by incubation of linoleic acid with soybean lipoxygenase at 0 °C, pH 9.0, under continuous oxygen bubbling followed by purification by NP-HPLC. (9*S*,10*E*,12*Z*)-9-Hydroperoxy-10,12-octadecadienoic acid (9-HPOD) was prepared by incubation of linoleic acid with tomato fruit lipoxygenase at 0 °C, pH 6.0, under the continuous oxygen bubbling followed by purification by NP-HPLC.

4.3. Cell-free preparations from Lily-of-the-Valley roots

Cut roots (10 g) of Lily-of-the-Valley were suspended in cold (0–4 °C) 0.1 M Tris/HCl buffer (20 ml, pH 7.5) and homogenized with Ultra-Turrax. Homogenate was filtered through cheesecloth and centrifuged at 15,000 *g* for 15 min. The supernatant was decanted and used for incubations with linoleic acid or its hydroperoxides.

Alternatively, the 15,000 *g* supernatant was adjusted to 70% of $(NH_4)_2SO_4$ saturation, stirred for 15 min and centrifuged at 15,000 *g* for 15 min. The supernatant was decanted and the pellet obtained after the addition of $(NH_4)_2SO_4$ was frozen with liquid nitrogen and stored at −85 °C prior use for incubations. Before use the preparation was unfrozen and suspended in 0.1 M Tris/HCl buffer, pH 7.5 at 0–4 °C. Aliquots of this suspension were used for incubations.

4.4. Incubation of enzyme preparations with linoleic acid and its hydroperoxides

Standard incubations were initiated by the addition of unlabelled linoleic acid (0.5 mg) to aliquot of 15,000 *g* supernatant preparation, corresponding to 5 g of the roots. The reaction mixture was stirred for 30 min under the continuous oxygen bubbling. The incubations aliquot of 15,000 *g* supernatant preparation, corresponding to 2 g of the roots with fatty acid hydroperoxides (0.5 mg of 13-HPOD, or 9-HPOD) were performed in the same way for 5 min, but without oxygen bubbling. For micro-preparative isolation of compounds **1** and **2** the 15,000 *g* supernatant obtained from 10 g of roots was incubated with 1 mg 13-HPOD or 9-HPOD.

Incubations with labelled substrates were performed as follows. Incubations were initiated by the addition of either [1-^{14}C]linoleic acid (74 KBq, 172 KBq/μmol) or 13-HPOD (74 KBq, 172 KBq/μmol) to an aliquot of suspension of $(NH_4)_2SO_4$ precipitate (corresponding to 0.3 g of the roots) in 1.8 ml of 0.1 M Tris/HCl buffer, pH 7.5. The reaction mixture was stirred at 23 °C for 10 min.

4.5. Extraction, preliminary purification and derivatization of products

After incubations the reaction mixtures were acidified with acetic acid to pH 4–5 followed by triple extraction, each with three volumes and extracted with ethyl acetate–hexane 1:1 (by volume). Acidic lipids were separated and purified for further analyses using the Supelclean LC-NH_2 (3 ml) cartridges (Supelco, Bellefonte, PA, USA) as described before (Grechkin et al., 2007). The total lipid extract dissolved in the solvent mixture chloroform–isopropanol 2:1 (by volume) was passed through the NH_2 cartridges. Then free carboxylic acids were eluted with solvent mixture ethyl acetate–acetic acid 98:2 (by volume). Free fatty acids of this fraction were esterified with diazomethane.

4.6. Analysis of products

The methyl esters of products (or their methyl ester TMSi derivatives) were subjected to direct GC–MS analyses after the amino cartridge purification and derivatization. Alternatively, the methyl esters of products were preliminarily separated by RP-HPLC and NP-HPLC (when specified). Products were separated as methyl esters by RP-HPLC on Macherey-Nagel Nucleosil 5 ODS column (250 × 4.6 mm) using the solvent mixture methanol–water (linear gradient from 76:24 to 96:4, by volume) at a flow rate of 0.4 ml/min. Products were collected and re-chromatographed by NP-HPLC on two serially connected Separon SIX columns (150 × 3.2 mm; 5 mm) eluted with hexane–diethyl ether 99.4:0.6 (by volume), flow rate 0.6 ml/min.

4.7. Spectral analyses

The UV spectra of isolated products were recorded with Varian Cary 50 spectrophotometer. Alternatively, the UV spectra of compounds that were purified by HPLC were recorded on-line by using an SPD-M20A diode array detector (Shimadzu). GC–MC analyses were performed by using a Shimadzu QP5050A mass spectrometer connected to Shimadzu. GC-17A gas chromatograph equipped with an MDN-5S (5% phenyl 95% methylpolysiloxane) fused capillary column (length, 30 m; i.d. 0.25 mm; film thickness, 0.25 μm). Helium at a flow rate of 30 cm/s was used as the carrier gas. Injections were made in the split mode by using an initial column temperature of 120 °C. The temperature was raised at 10 °C/min until 240 °C. Full scan or selected-ion monitoring (SIM) analyses were both performed by using ionization energy of 70 eV. The ^{1}H NMR and 2D-COSY spectra were recorded with Bruker Avance 400 instrument, 400 MHz, [$^2H_3$]acetonitrile, 296 K.

Acknowledgements

This work was supported by Grant 06-04-48430 from Russian Foundation for Basic Research and a Grant from Russian Academy of Sciences (program "Molecular and Cell Biology"). The authors appreciate the ^{1}H NMR and COSY records by Dr. Oleg I. Gnezdilov.

References

Blée, E., 1998. Phytooxylipins and plant defense reactions. Prog. Lipid Res. 37, 33–72.

Chechetkin, I.R., Blufard, A., Hamberg, M., Grechkin, A.N., 2008. A lipoxygenase-divinyl ether synthase pathway in flax (*Linum usitatissimum* L.) leaves. Phytochemistry 69, 2008–2015.

Cowley, T., Walters, D., 2005. Local and systemic effects of oxylipins on powdery mildew infection in barley. Pest Manag. Sci. 61, 572–576.

Fammartino, A., Cardinale, F., Göbel, C., Mène-Saffrané, L., Fournier, J., Feussner, I., Esquerré-Tugayé, M.-T., 2007. Characterization of a divinyl ether biosynthetic pathway specifically associated with pathogenesis in tobacco. Plant Physiol. 143, 378–388.

Galliard, T., Phillips, D.R., 1972. The enzymatic conversion of linoleic acid into 9-(nona-1,3-dienoxy)-non-8-enoic acid, a novel unsaturated ether derivative isolated from homogenates of *Solanum tuberosum* tubers. Biochem. J. 129, 743–753.

Galliard, T., Phillips, D.R., Frost, D.J., 1973. Novel divinyl ether fatty acids in extracts of *Solanum tuberosum*. Chem. Phys. Lipids 11, 173–180.

Galliard, T., Mathew, J.A., 1975. Enzymic reactions of fatty acid hydroperoxides in extracts of potato tuber. I. Comparison 9D- and 13L-hydroperoxyoctadecadienoic acids as substrates for the formation of a divinyl ether derivative. Biochim. Biophys. Acta 398, 1–9.

Göbel, C., Feussner, I., Schmidt, A., Scheel, D., Sanchez-Serrano, J., Hamberg, M., Rosahl, S., 2001. Oxylipin profiling reveals the preferential stimulation of the 9-lipoxygenase pathway in elicitor-treated potato cells. J. Biol. Chem. 276, 6267–6273.

Granér, G., Hamberg, M., Meijer, J., 2003. Screening of oxylipins for control of oilseed rape (*Brassica napus*) fungal pathogens. Phytochemistry 63, 89–95.

Grechkin, A.N., 1998. Recent developments in biochemistry of the plant lipoxygenase pathway. Prog. Lipid Res. 37, 317–352.

Grechkin, A.N., 2002. Hydroperoxide lyase and divinyl ether synthase. Prostag. Other Lipid Mediat. 68–69, 457–470.

Grechkin, A.N., Fazliev, F.N., Mukhtarova, L.S., 1995. The lipoxygenase pathway in garlic (*Allium sativum* L.) bulbs: detection of the novel divinyl ether oxylipins. FEBS Lett. 371, 159–162.

Grechkin, A.N., Hamberg, M., 1996. Divinyl ether synthase from garlic (*Allium sativum* L.) bulbs: subcellular localization and substrate regio – and stereospecificity. FEBS Lett. 388, 112–114.

Grechkin, A.N., Ilyasov, A.V., Hamberg, M., 1997. On the mechanism of biosynthesis of divinyl ether oxylipins by enzyme from garlic bulbs. Eur. J. Biochem. 245, 137–142.

Grechkin, A.N., Ogorodnikova, A.V., Gnezdilov, O.I., Mukhtarova, L.S., 2007. Detection of a pathway from linoleate to a novel cyclopentenone: *cis*-12-oxo-10-phytoenoic acid in sunflower roots. Chem. BioChem. 8, 2275–2280.

Hamberg, M., 1998. A pathway for biosynthesis of divinyl ether fatty acids in green leaves. Lipids 33, 1061–1071.

Hamberg, M., 2002. Biosynthesis of new divinyl ether oxylipins in *Ranunculus* plants. Lipids 37, 427–433.

Hamberg, M., 2004. Isolation and structures of two divinyl ether fatty acids from *Clematis vitalba*. Lipids 39, 565–569.

Hamberg, M., 2005. Hidden stereospecificity in the biosynthesis of divinyl ether fatty acids. FEBS J. 272, 736–743.

Itoh, A., Howe, G.A., 2001. Molecular cloning of a divinyl ether synthase. Identification as a CYP74 cytochrome P450. J. Biol. Chem. 276, 3620–3627.

Jiang, Z.D., Gerwick, W.H., 1997. Novel oxylipins from the temperate red alga *Polyneura latissima*: evidence for an arachidonate 9(*S*)-lipoxygenase. Lipids 32, 231–235.

Proteau, P.J., Gerwick, W.H., 1993. Divinyl ethers and hydroxy fatty acids from three species of *Laminaria* (brown algae). Lipids 28, 783–787.

Stumpe, M., Carsjens, J.-G., Göbel, C., Feussner, I., 2008. Divinyl ether synthesis in garlic bulbs. J. Exp. Bot. 59, 907–915.

Stumpe, M., Feussner, I., 2006. Formation of oxylipins by CYP74 enzymes. Phytochem. Rev. 5, 347–357.

Stumpe, M., Kandzia, R., Göbel, C., Rosahl, S., Feussner, I., 2001. A pathogen-inducible divinyl ether synthase (CYP74D) from elicitor-treated potato suspension cells. FEBS Lett. 507, 371–376.

Weber, H., Chetalat, A., Caldelari, D., Farmer, E.E., 1999. Divinyl ether fatty acid synthesis in late blight-diseased potato leaves. Plant Cell 11, 485–494.