ARTICLE

New Cyclopentenone Fatty Acids Formed from Linoleic and Linolenic Acids in Potato

Mats Hamberg*

Department of Medical Biochemistry and Biophysics, Division of Physiological Chemistry II, Karolinska Institutet, S-171 77 Stockholm, Sweden

ABSTRACT: [1-^{14}C]Linoleic acid was incubated with a whole homogenate preparation from potato stolons. The reaction product contained four major labeled compounds, i.e., the α-ketol 9-hydroxy-10-oxo-12(*Z*)-octadecenoic acid (59%), the epoxy alcohol 10(*S*),11(*S*)-epoxy-9(*S*)-hydroxy-12(*Z*)-octadecenoic acid (19%), the divinyl ether colneleic acid (3%), and a new cyclopentenone (13%). The structure of the last-mentioned compound was determined by chemical and spectral methods to be 2-oxo-5-pentyl-3-cyclopentene-1-octanoic acid (trivial name, 10-oxo-11-phytoenoic acid). Steric analysis demonstrated that the relative configuration of the two side chains attached to the five-membered ring was *cis,* and that the compound was a racemate comprising equal parts of the 9(*R*),13(*R*) and 9(*S*),13(*S*) enantiomers. Experiments in which specific trapping products of the two intermediates 9(*S*)-hydroperoxy-10(*E*),12(*Z*)-octadecadienoic acid and 9(*S*),10-epoxy-10,12(*Z*)-octadecadienoic acid were isolated and characterized demonstrated the presence of 9-lipoxygenase and allene oxide synthase activities in the tissue preparation used. The allene oxide generated from linoleic acid by action of these enzymes was further converted into the cyclopentenone and α-ketol products by cyclization and hydrolysis, respectively. Incubation of [1-^{14}C]linolenic acid with the preparation of potato stolons afforded 2-oxo-5-[2′(*Z*)-pentenyl]-3-cyclopentene-1-octanoic acid (trivial name, 10-oxo-11,15(*Z*)-phytodienoic acid), i.e., an isomer of the jasmonate precursor 12-oxo-10,15(*Z*)-phytodienoic acid. Quantitative determination of 10-oxo-11-phytoenoic acid in linoleic acid-supplied homogenates of different parts of the potato plant showed high levels in roots and stolons, lower levels in developing tubers, and no detectable levels in leaves.

Paper no. L8431 in *Lipids 35,* 353–363 (April 2000).

*E-mail: Mats.Hamberg@mbb.ki.se

Abbreviations and trivial names: 9-H(P)OD, 9-hydro(pero)xy-10(*E*),12(*Z*)-octadecadienoic acid; 9-H(P)OT, 9-hydro(pero)xy-10(*E*),12(*Z*),15(*Z*)-octadecatrienoic acid; 12-oxo-PDA, 12-oxo-10,15(*Z*)-phytodienoic acid {4-oxo-5-[2′(*Z*)-pentenyl]-2-cyclopentene-1-octanoic acid}; 12-oxo-PEA, 12-oxo-10-phytoenoic acid [4-oxo-5-pentyl-2-cyclopentene-1-octanoic acid]; 13-H(P)OD, 13-hydro(pero)xy-9(*Z*),11(*E*)-octadecadienoic acid; 13-H(P)OT, 13-hydro(pero)xy-9(*Z*),11(*E*),15(*Z*)-octadecatrienoic acid; AOS, allene oxide synthase; colneleic acid, 9-[1′(*E*),3′(*Z*)-nonadienyloxy]-8(*E*)-nonenoic acid; colnelenic acid, 9-[1′(*E*),3′(*Z*),6′(*Z*)-nonatrienyloxy]-8(*E*)-nonenoic acid; CP-HPLC, chiral-phase high-performance liquid chromatography; FTIR, Fourier transform infrared; GC–MS, gas chromatography–mass spectrometry; GLC, gas–liquid chromatography; GSH, reduced glutathione; GSH-px, glutathione peroxidase; MC, (−)-menthoxycarbonyl; NMR, nuclear magnetic resonance; RP-HPLC, reversed-phase high-performance liquid chromatography; SP-HPLC, straight-phase high-performance liquid chromatography; UV, ultraviolet.

Jasmonates constitute a group of biologically important compounds in plants. Members of the jasmonate family have been implicated in defense reactions against insects and other pathogens, in mechanical responses such as tendril coiling, and in pollen development (1). Jasmonic acid is biosynthesized from the cyclopentenone 12-oxo-10,15(*Z*)-phytodienoic acid (12-oxo-PDA) by action of reductase and β-oxidation enzymes (2). 12-Oxo-PDA is formed by a pathway involving 13-lipoxygenase, allene oxide synthase, and allene oxide cyclase (3,4). The last-mentioned enzyme is specific for allene oxides in which the epoxide group is located in the n-6,7 position and in which there is also a double bond in the n-3 position (5). In agreement with these structural requirements, cyclopentenone fatty acids hitherto isolated from higher plants originate in linolenic acid (1,4) or 7(*Z*),10(*Z*),13(*Z*)-hexadecatrienoic acid (6). The present paper is concerned with the oxidative metabolism of linoleic and linolenic acids in preparations of stolons of potato. An alternative route to cyclopentenone fatty acids initiated by a 9-lipoxygenase is described.

EXPERIMENTAL PROCEDURES

Plant materials. Tubers of potato (*Solanum tuberosum* L., var. Bintje) were stored in the dark at 18°C. Stolons of 0.5–5 cm length were used for the incubations.

Fatty acids. Linoleic and linolenic acids were purchased from Nu-Chek-Prep (Elysian, MN). [1-^{14}C]Linoleic and [1-^{14}C]linolenic acids (DuPont NEN, Boston, MA) were mixed with the corresponding unlabeled acids and purified by SiO_2 chromatography to afford specimens having specific radioactivities of 5.4 and 9.1 kBq/μmol, respectively. [1-^{14}C]9(*S*)-Hydroperoxy-10(*E*),12(*Z*)-octadecadienoic acid [9(*S*)-HPOD] and [1-^{14}C]9(*S*)-hydroperoxy-10(*E*),12(*Z*),15(*Z*)-octadecatrienoic acid [9(*S*)-HPOT] were prepared in 30–50% yield by incubation of labeled linoleic acid and linolenic acid, respectively, with tomato lipoxygenase (7) under an atmosphere of oxygen gas. The α-ketol 9-hydroxy-10-oxo-12(*Z*)-octadecenoic acid and the γ-ketol 13-hydroxy-10-oxo-11(*E*)-octadecenoic acid were prepared by incubation of 9(*S*)-HPOD with allene oxide synthase (AOS) from corn seeds (8,9) followed by isolation by reversed-phase high-performance liquid chromatography (RP-HPLC). 9-Methoxy-10-oxo-12(*Z*)-octadecenoic acid was prepared from 9(*S*)-HPOD in brief incu-

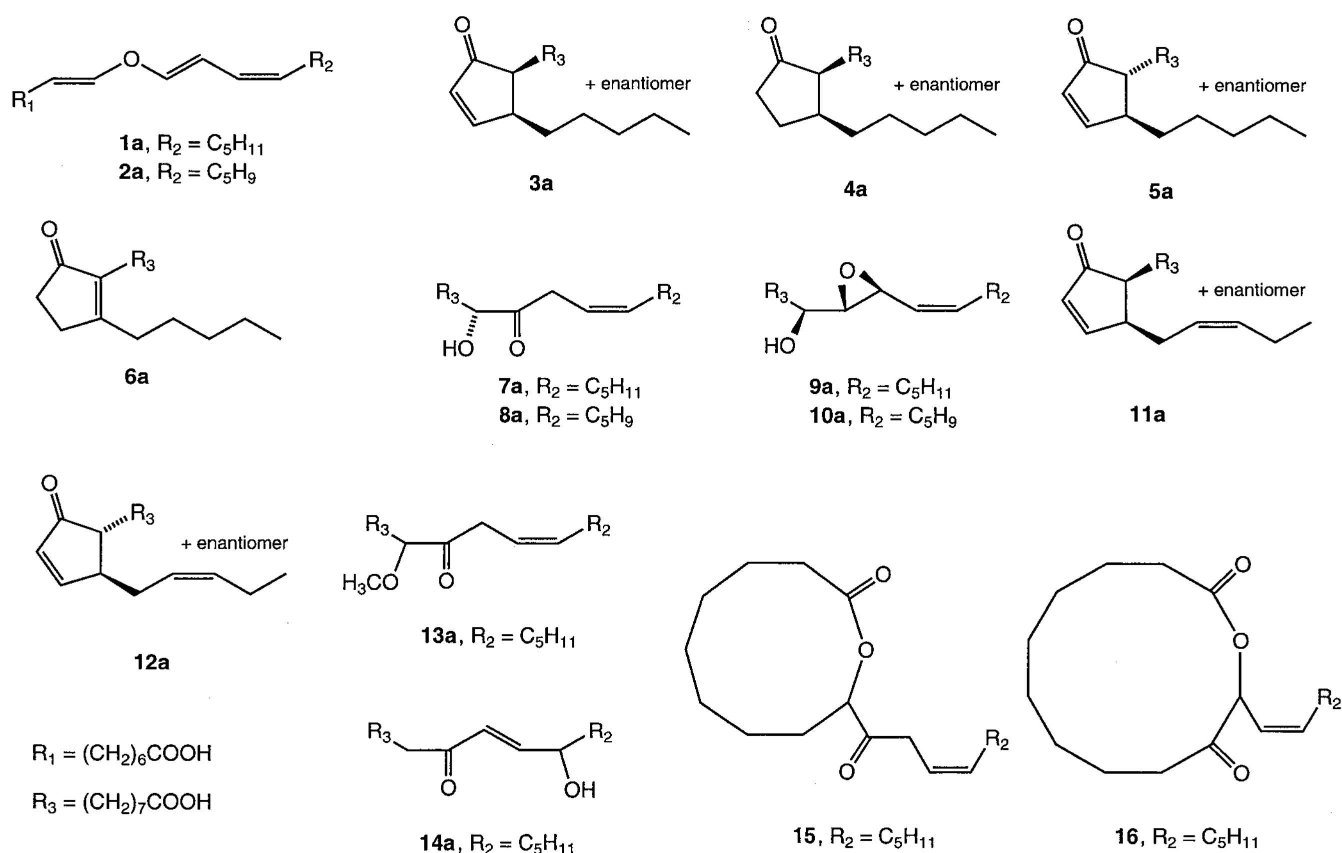
Copyright © 2000 by AOCS Press

bations with corn seed AOS interrupted by addition of 20 vol of methanol (8,9). The macrolactones 10-oxo-12(*Z*)-octadecen-9-olide and 10-oxo-12(*Z*)-octadecen-11-olide were obtained in a similar way in incubations interrupted by addition of 20 vol of acetonitrile (9,10). Incubation of 13(*S*)-hydroperoxy-9(*Z*),11(*E*),15(*Z*)-octadecatrienoic acid [13(*S*)-HPOT] with corn seed AOS in the presence of corn allene oxide cyclase afforded natural 9(*S*),13(*S*)-12-oxo-10,15(*Z*)-phytodienoic acid [9(*S*),13(*S*)-12-oxo-PDA] (11), whereas 12-oxo-10-phytoenoic acid [12-oxo-PEA (side-chain *trans* form)] was obtained by brief incubation of 13(*S*)-hydroperoxy-9(*Z*),11(*E*)-octadecadienoic acid [13(*S*)-HPOD] with corn seed AOS followed by addition of bovine serum albumin (12). The methyl ester of the epoxy alcohol 10(*S*),11(*S*)-epoxy-9(*S*)-hydroxy-12(*Z*)-octadecenoic acid was obtained by incubation of 9(*S*)-HPOD with a homogenate of potato leaves (13), whereas the divinyl ethers colneleic acid and colnelenic acid were prepared by incubation of 9(*S*)-HPOD and 9(*S*)-HPOT, respectively, with a preparation from potato tubers (14).

Enzyme preparations. Stolons of potato were minced and homogenized at 0°C in 0.1 M potassium phosphate buffer pH 7.4 (1:10, wt/vol) with an Ultra-Turrax. The homogenate was filtered through gauze, and the filtrate (protein, 1.3 mg/mL) was used as enzyme source in preparative incubations carried out to generate products for structural analysis. Centrifugation at 9300 × *g* for 15 min afforded a low-speed sediment and a supernatant. Further centrifugation of the latter at 105,000 × *g* for 60 min provided a high-speed particle fraction and a particle-free supernatant (protein, 0.9 mg/mL). The former was resuspended in buffer to give a suspension (protein, 0.8 mg/mL), which was used as enzyme source in experiments carried out to study conditions affecting formation of cyclopentenones. A particle fraction containing AOS from corn seeds was prepared as described (15). Glutathione peroxidase (GSH-px) and reduced glutathione (GSH) were purchased from Sigma Chemical Co. (St. Louis, MO).

Incubations and treatments. Incubations of [1-^{14}C]linoleic acid, [1-^{14}C]linolenic acid, 9(*S*)-HPOD and 9(*S*)-HPOT (63–245 μM as indicated) were carried out by stirring the substrate at 23°C for times indicated with the whole homogenate preparation of potato stolons. The mixtures were extracted with 2 vol of diethyl ether at pH 5, and the products were methyl-esterified and subjected to straight-phase high-performance liquid chromatography (SP-HPLC) [solvent system, 2-propanol/hexane (1:99, vol/vol)]. Cyclopentenone formation under various conditions was studied by stirring [1-^{14}C]9(*S*)-HPOD at 23°C with a suspension of the 105,000 × *g* particle fraction of potato stolons (2 mL, pre-warmed at 23°C for 5 min). This incubation was modified as indicated with respect to temperature, pH, and substrate concentration.

*Preparation of **3a***. For large-scale preparation of cyclopentenone **3a** (see Scheme 1 for numbered key to all struc-



SCHEME 1

tures; free carboxylic acids are designated by **a** and methyl esters by **b**), batches of 13 g of potato stolons were minced and homogenized in 130 mL of 0.1 M potassium phosphate buffer pH 7.4 at 0°C. The homogenate was filtered through gauze, and 110 mL of the filtrate was diluted with 400 mL of potassium phosphate buffer and warmed at 26°C for 5 min. Linoleic acid (245 μM) was added, and the mixture was stirred at 26°C for 30 min. The material obtained by extraction with diethyl ether was subjected to RP-HPLC using a column of Nucleosil C_{18} 100-7 (250 × 10 mm) purchased from Macherey-Nagel (Düren, Germany) and a solvent system consisting of acetonitrile/water/2 M hydrochloric acid (55:45:0.013, by vol; 4 mL/min). The material collected was subjected to SP-HPLC using a column of Nucleosil 50-7 (250 × 10 mm) and a solvent system of 2-propanol/hexane/acetic acid (1.5:98.5:0.01, by vol; 4 mL/min). This procedure afforded **3a** as a colorless oil in 2–4% overall yield.

*Quantitative determination of **3a** in potato plant homogenates supplied with linoleic acid*. Different tissue types (2 g) from the potato plant were placed in ice-cold potassium phosphate buffer pH 7.4 (20 mL) and homogenized with an Ultra-Turrax. The homogenate was filtered through gauze, and an aliquot of the filtrate (5 mL) was prewarmed at 23°C for 5 min. Linoleic acid or 9(*S*)-HPOD (both 200 μM) was added, and the mixture was stirred for 10 min at 23°C. Ethanol (15 mL) containing 12-oxostearic acid (51.5 μg) was added, and the mixture was extracted with diethyl ether. Aliquots of the methyl-esterified material were directly subjected to gas chromatography–mass spectrometry (GC–MS) operated in the selected ion monitoring mode. The ions *m/z* 95 and 152 (typical for **3b**) and *m/z* 242 and 281 (typical for methyl 12-oxostearate) were used. The amounts of **3a** were calculated from the ratio of intensities of ions (*m/z* 95 + 152)/(*m/z* 242 + 281) and a standard curve constructed by analyzing mixtures of **3a** and 12-oxostearic acid in known proportions.

Chemical methods. Catalytic hydrogenation, oxidation with potassium permanganate, oxidative ozonolysis, and derivative preparation for GC–MS were performed as described earlier (13,16). Preparation of diastereomeric derivatives of cyclopentenones and steric analysis of these by gas–liquid chromatography (GLC) were carried out using a published procedure (15). Methyl-esterification was performed by brief (*ca.* 10 s) treatment with ethereal diazomethane.

Chromatographic and instrumental methods. The equipment and conditions used for RP-HPLC, SP-HPLC, GLC, GC–MS, ultraviolet (UV), and Fourier transform infrared (FTIR) spectrometry have been described in detail (13,16). Chiral phase HPLC (CP-HPLC) was carried out with a Chiralcel OB-H column (250 × 4.6 mm) purchased from Daicel Chemical Industries (Osaka, Japan) using mixtures of 2-propanol/hexane as the solvent and a flow rate of 0.5 mL/min. Nuclear magnetic resonance (NMR) spectra were recorded with a JEOL JNM-EX270 instrument. Deuteriochloroform containing 0.03% tetramethylsilane was used as the solvent.

RESULTS

Isolation and structure determination of oxidation products. (i) Incubation of linoleic acid. [1-^{14}C]Linoleic acid (200 μM) was stirred at 23°C for 10 min with whole homogenate of potato stolons, and the methyl-esterified product was subjected to SP-radio-HPLC. As seen in Figure 1A, four major radioactive compounds appeared, i.e., **1b** (3% of the recovered radioactivity; effluent volume, 2.5 mL), **3b** (13%; 6.3 mL), **7b** (59%; 7.5 mL), and **9b** (19%; 9.7 mL).

*(ii) Identification of compound **1b***. The UV spectrum of **1b** showed an absorption band with λ_{max} = 250 nm, suggesting a fatty acid divinyl ether derivative (14,16). The C-value (*cf.* Ref. 13), i.e., 19.41, and the mass spectrum were identical to those of methyl colneleate, a lipoxygenase product formed in tubers of potato (14). Results of catalytic hydrogenation and oxidative ozonolysis performed on compound **1b** (*cf.* Refs. 14,16) conclusively established its identity to methyl colneleate.

*(iii) Identification of compound **7b***. The UV spectrum of **7b** was featureless, whereas the FTIR spectrum showed strong absorption bands at 3480 (OH), 1739 (ester C=O), and 1716 cm^{-1} (ketone C=O). No significant absorption band was observed in the region 900–1000 cm^{-1}, thus excluding the presence of a *trans* double bond. The Me_3Si derivative of **7b** gave a C-value of 20.93 on GLC analysis, and the mass spectrum showed prominent ions at *m/z* 383 (3%; M^+ – 15; loss of $\cdot CH_3$), 259 [100; Me_3SiO^+=CH–$(CH_2)_7$–$COOCH_3$], 155 [37; OHC–$(CH_2)_7$–C≡O^+], 129 (9; Me_3SiO^+=CH–CH=CH_2), 109 (19), and 73 (47; Me_3Si^+). Identical results were obtained upon analysis of the methyl ester/Me_3Si derivative of 9-hydroxy-10-oxo-12(*Z*)-octadecenoic acid, an α-ketol fatty acid which is formed as the major product upon incubation of 9(*S*)-HPOD with corn seed AOS (9). An aliquot of **7b** (0.5 mg) was treated with sodium borohydride in methanol and subsequently subjected to catalytic hydrogenation. The product was identified by GLC and GC–MS as methyl 9,10-dihydroxystearate (mixture of *erythro*- and *threo*-isomers; authentic reference compounds were prepared by *cis*- and *trans*-hydroxylation, respectively, of methyl oleate). In another experiment, compound **7b** (1 mg) was derivatized with (–)-menthoxycarbonyl (MC) chloride, and the resulting MC derivative was subjected to $KMnO_4$ oxidation. The methyl-esterified product consisted of a main chiral fragment, i.e., the MC derivative of dimethyl 2-hydroxy-1,10-decanedioate. Steric analysis by GLC revealed that 92% of the sample was due to the 2(*R*)-hydroxy derivative and 8% 2(*S*)-hydroxy derivative. Steric analysis of compound **7b** also could be effected by resolution by CP-HPLC [solvent system, 2-propanol/hexane (6:94, vol/vol)]. Peaks due to the *R* (effluent volume, 10.0 mL) and *S* (9.0 mL) enantiomers in a 92:8 ratio were observed. Accordingly, **7b** was a mixture containing 92% methyl 9(*R*)-hydroxy-10-oxo-12(*Z*)-octadecenoate and 8% methyl 9(*S*)-hydroxy-10-oxo-12(*Z*)-octadecenoate.

*(iv) Identification of compound **9b***. Analysis of the Me_3Si derivative of **9b** by GC–MS showed a C-value (20.86) and a mass spectrum identical to those of the corresponding deriva-

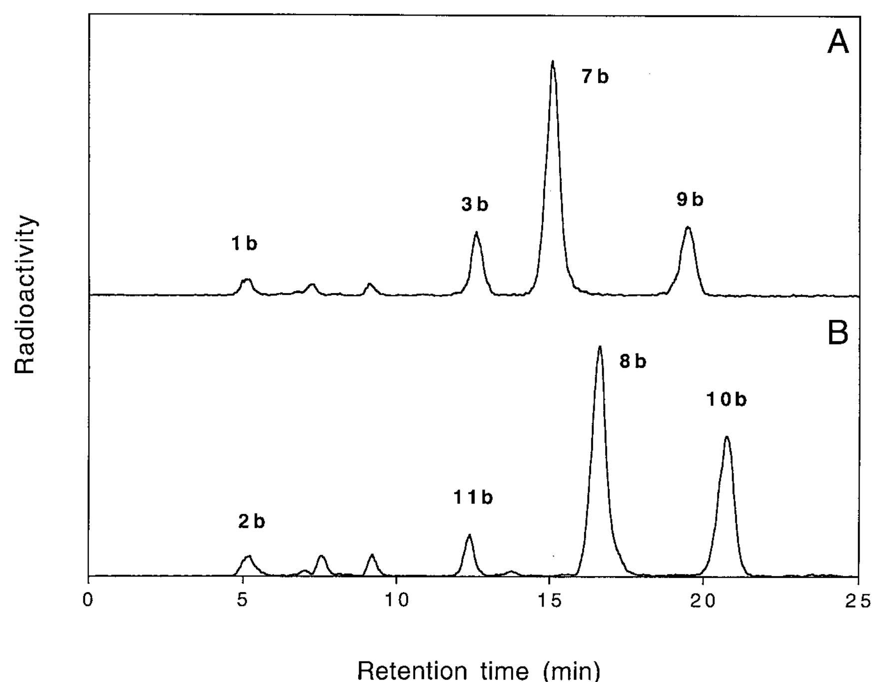


FIG. 1. Analysis by straight-phase radio high-performance liquid chromatography of methyl-esterified reaction products obtained following incubations of [1-^{14}C]linoleic acid (200 µM; panel A) and [1-^{14}C]linolenic acid (200 µM; panel B) at 23°C for 10 min with whole homogenate of stolons of potato. The column was eluted at a flow rate of 0.5 mL/min with 2-propanol/hexane (1:99, vol/vol). The structures of the compounds indicated are given in Scheme 1.

tive of methyl 10(*S*),11(*S*)-epoxy-9(*S*)-hydroxy-12(*Z*)-octadecenoate, an epoxy alcohol recently isolated following incubation of linoleic acid with a preparation of potato leaves (13). Further support for the identity of **9b** with this epoxy alcohol was provided by catalytic hydrogenation, which afforded methyl *erythro*-9,10-dihydroxystearate, and by results of mild acid-catalyzed hydrolysis, which afforded methyl 9(*S*),10(*R*),13(*R*)-trihydroxy-11(*E*)-octadecenoate and methyl 9(*S*),10(*R*),13(*S*)-trihydroxy-11(*E*)-octadecenoate as the main products (13).

(v) Structure of compound ***3b***. The UV spectrum of **3b** showed a strong absorption band with λ_{max} = 220 nm (solvent, ethanol) (Table 1), and the FTIR spectrum showed carbonyl absorption bands at 1739 (ester C=O) and 1709 cm^{-1} (ring ketone C=O) (Fig. 2). The mass spectrum showed ions at *m/z* 308 (3%; M^+), 277 (9; $M^+ - 31$; loss of $\cdot OCH_3$), 237 [2; M^+ - 71; loss of $\cdot(CH_2)_4CH_3$], 233 (3), 192 (5), 152 [93; $M^+ - 156$; cleavage β to ring carbonyl and elimination of CH_2=CH–$(CH_2)_5COOCH_3$], 123 (11), 109 (20), 95 [100; C_6H_7O; tentatively ascribed to loss of $\cdot(CH_2)_3CH_3$ from *m/z* 152], and 82 [34; C_5H_6O; loss of CH_2=CH–$(CH_2)_2CH_3$ from *m/z* 152]. These spectral data suggested that **3b** was built up by a cyclopentenone ring carrying two side chains, i.e., C_5H_{11} and C_7H_{14}–$COOCH_3$. Signals observed in the NMR spectrum of **3b** are given in Table 2. Protons belonging to a pentyl side chain (H14 to H18; 11 protons) and to a carboxylic ester side chain (H2 to H8; 14 protons) were readily discerned. Olefinic protons that were part of a cyclopentenone structure (H11 and H12; 2 protons) appeared at 6.15 and 7.71 ppm, whereas the protons at the side chain bearing carbons (H9 and H13; 2 protons) gave rise to signals at 2.32 and 2.96 ppm. The chemical shifts of the two last-mentioned signals were similar to those previously recorded for the H13 and H9 protons, respectively, of the methyl ester of natural 12-oxo-PDA (17), thus indicating a *cis* relationship between the two side chains in **3b**. Conclusive evidence for the *cis* relative configuration was provided by the alkali-promoted conversion of **3b** into the side-

TABLE 1
Ultraviolet and Gas Chromatographic Data of Cyclopentenones

Compound	λ_{max}[a] (nm)	C-value[b]
3b	220	20.17
4b	—	19.87
5b	221	19.77
6b	237	20.37
11b	217	20.11
12b	217	19.75

[a]Ultraviolet spectra were recorded on compounds dissolved in 99.5% ethanol.
[b]Gas–liquid chromatography was performed using a methylsilicone capillary column (25 m) operated at 230°C. Abbreviations: **3b**, 2-oxo-5-pentyl-3-cyclopentene-1-octanoic acid (1,5-*cis* isomer, methyl ester); **4b**, 2-oxo-5-pentylcyclopentane-1-octanoic acid (1,5-*cis* isomer, methyl ester); **5b**, 2-oxo-5-pentyl-3-cyclopentene-1-octanoic acid (1,5-*trans*-isomer, methyl ester); **6b**, 2-oxo-5-pentyl-1(5)-cyclopentene-1-octanoic acid (methyl ester); **11b**, 2-oxo-5-[2′(*Z*)-pentenyl]-3-cyclopentene-1-octanoic acid (1,5-*cis* isomer; methyl ester); **12b**, 2-oxo-5-[2′(*Z*)-pentenyl]-3-cyclopentene-1-octanoic acid (1,5-*trans* isomer; methyl ester).

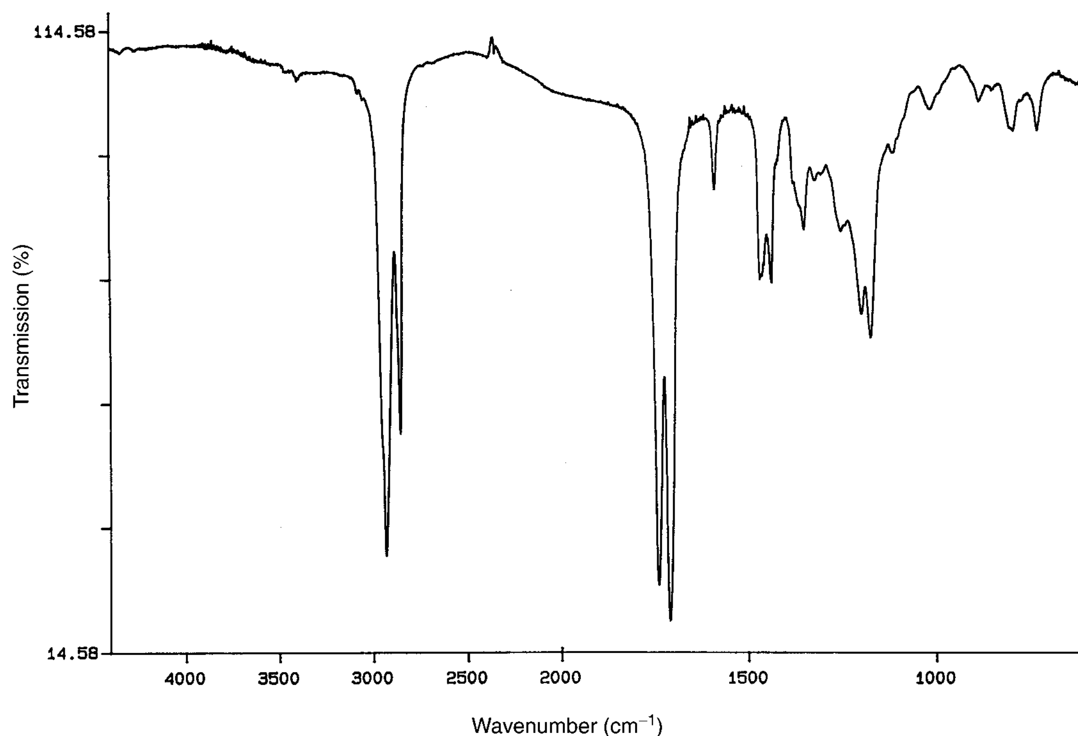


FIG. 2. Fourier transform infrared spectrum (film) of compound **3b**, (±)-2-oxo-5-pentyl-3-cyclopentene-1-octanoic acid, methyl ester.

chain *trans* isomer **5b**, and the NMR spectrum of this compound (see below).

Independent support for the structure of **3b** was provided by an experiment in which a sample (0.5 mg) was subjected to oxidative ozonolysis. The methyl-esterified product was analyzed by GC–MS and found to be due to a major fragment identified as trimethyl tetradecane-1,8,9-tricarboxylate by its mass spectrum, i.e., *m/z* 341 (63%; $M^+ - 31$; loss of $\cdot OCH_3$), 308 (12; $M^+ - 2 \times 32$), 270 [26; $M^+ - (70 + 32)$; loss of $CH_2{=}CH{-}(CH_2)_2CH_3$ plus CH_3OH], 216 [22; $M^+ - 156$; loss of $CH_2{=}CH{-}(CH_2)_5COOCH_3$], 184 (37; *m/z* 216 – 32), 152 (46; *m/z* 216

TABLE 2
Proton Nuclear Magnetic Resonance Data for Compounds 3b and 5b[a]

	Compound **3b**			Compound **5b**		
Carbon #	δ (ppm)	Multiplicity	*J* (Hz)	δ (ppm)	Multiplicity	*J* (Hz)
2	2.31	*t*	7.4	2.30	*t*	7.4
3	1.63	*m*		1.62	*m*	
4	1.28–1.36	*m*		1.27–1.35	*m*	
5	1.28–1.36	*m*		1.27–1.35	*m*	
6	1.28–1.36	*m*		1.27–1.35	*m*	
7	1.28–1.36	*m*		1.27–1.35	*m*	
8	1.38–1.48	*m*		—[b]		
9	2.32	*m*		1.94	*m*	
11	6.15	*dd*	5.9, 1.8	6.10	*dd*	5.7,1.8
12	7.71	*dd*	5.9, 2.7	7.60	*dd*	5.7, 2.5
13	2.96	*m*		2.58	*m*	
14	≈1.15, ≈1.70	*m*		—[b]		
15	1.28–1.36	*m*		1.27–1.35	*m*	
16	1.28–1.36	*m*		1.27–1.35	*m*	
17	1.28–1.36	*m*		1.27–1.35	*m*	
18	0.90	*t*	6.6	0.90	*t*	6.7
OCH_3	3.67	*s*		3.66	*s*	

[a]Proton nuclear magnetic resonance spectra were recorded at 270 MHz in $CDCl_3$ with tetramethylsilane as internal chemical shift reference.
[b]The absorptions due to H-8 and H-14 were hidden in other absorption bands and could not be adequately resolved. For abbreviations see Table 1.

– 2 × 32), and 55 (100). Formation of this product involved cleavage of the double bond of the five-membered ring followed by oxidative decarboxylation of the resulting 2-oxoacid.

The absolute stereochemistry of **3b** was studied in two ways. In one set of experiments, previously developed methodology for steric analysis of 12-oxo-PDA (11,15) and 12-oxo-PEA (12) involving reduction into epimeric cyclopentanols and derivatization of these with MC chloride was applied to a sample of **3b**. Analysis of the diastereomeric MC derivatives showed two peaks in a ratio of 50:50 corresponding to the 9(*R*),13(*R*) and 9(*S*),13(*S*) enantiomers of **3b**. In another experiment, **3b** was subjected to CP-HPLC using a solvent system of 2-propanol/hexane (1:9, vol/vol). Two peaks (8.6 and 10.6 mL effluent) appeared in a ratio of 50:50 (49:51 – 51:49 in different runs). On the basis of these results, compound **3a** was assigned the structure (±)-2-oxo-5-pentyl-3-cyclopentene-1-octanoic acid. The trivial name of **3a** will be 10-oxo-11-phytoenoic acid using the phytonoic acid (18) stem.

*(vi) Preparation and analysis of **5b***. Compound **3b** (4 mg) was treated with 0.1 M NaOH in 90% aqueous methanol at 23°C for 30 min. The reesterified product was purified by SP-HPLC to yield compound **5b**. Analysis of this material by UV spectroscopy showed an absorption band with λ_{max} (EtOH) = 221 nm. The FTIR spectrum showed carbonyl absorption bands at 1739 (ester C=O) and 1708 cm^{-1} (ring ketone C=O), and the mass spectrum was similar to that of **3b**. As seen in Table 1, the C-value of **5b** (19.77) was significantly lower than that of **3b** (20.17), suggesting that **5b** was a side-chain *trans* isomer (*cf.* Refs. 12,19). Conclusive evidence for the notion that alkali-treatment of **3b** resulted in reversible enolization at C-9 and formation of the thermodynamically more stable *trans* isomer **5b** was provided by the NMR spectrum of **5b** (Table 2). As seen, the protons attached to C-9 and C-13 gave rise to signals (1.94 and 2.58 ppm, respectively) that were shifted upfield compared to the corresponding signals of **3b** (2.32 and 2.96 ppm, respectively). Such an upfield shift is expected for a vicinal dialkylcyclopentane derivative in which the carbon chains are oriented *syn* with respect to the vicinal ring juncture proton (20), and therefore demonstrated that in the pair **3b** and **5b** the latter is the *trans* and the former is the *cis* side-chain stereoisomer. The NMR spectra of **3b** and **5b** were analogous to those earlier recorded on the *cis*- and *trans*-forms, respectively, of the methyl ester of 12-oxo-PDA (17).

*(vii) Preparation and analysis of **6b***. Compound **3b** (1 mg) was treated with 0.5 M NaOH in 50% aqueous methanol at 70°C for 1 h. The product was methyl-esterified and purified by SP-HPLC to afford **6b**. The UV spectrum of this compound showed a strong absorption band with λ_{max} (EtOH) = 237 nm, and the FTIR spectrum showed carbonyl absorption bands at 1739 (ester C=O) and 1699 cm^{-1} (ring ketone C=O) as well as an olefinic absorption band at 1640 cm^{-1} (C=C stretching of tetrasubstituted double bond conjugated to ring ketone). Analysis by GC–MS revealed a C-value of 20.37 and a mass spectrum showing prominent ions at *m/z* 308 (50%; M^+), 276 (37; M^+ – 32; loss of CH_3OH), 237 [59; M^+ – 71; loss of $\cdot(CH_2)_4CH_3$], 233 [100; M^+ – (43 + 32); loss of $\cdot(CH_2)_2CH_3$ plus CH_3OH], 205 (38; *m/z* 237 – 32), 123 (79), and 110 (93).

Oxidative ozonolysis performed on cyclopentenone **6b** followed by methyl-esterification afforded comparable amounts of dimethyl 1,9-nonanedioate and methyl 4-oxononanoate. The latter compound was identified by its mass spectrum, which showed prominent ions at *m/z* 155 (20%; M^+ – 31; loss of $\cdot OCH_3$), 130 (59; M^+ – 56; β-cleavage with loss of CH_2=CH–CH_2CH_3), 115 [67; M^+ – 71; α-cleavage with loss of $\cdot(CH_2)_4CH_3$], 98 (100; *m/z* 130 – 32), 71 (57), and 55 (75). The ozonolysis fragments were likely formed from **6b** by initial cleavage of the tetrasubstituted double bond followed by oxidative cleavage of the resulting α-dioxo structure.

*(viii) Preparation and analysis of **4b***. Compound **3b** (0.5 mg) was hydrogenated using platinum catalyst to provide dihydro derivative **4b** (C-value, 19.87) as well as a smaller amount of the corresponding side-chain *trans* derivative (C-value, 19.60). The mass spectra recorded on these compounds were similar and showed a molecular ion at *m/z* 310 (1%), as well as prominent ions at *m/z* 154 [18; M^+ – 156; β-cleavage with loss of CH_2=CH–$(CH_2)_5COOCH_3$], and 83 [100; C_5H_7O; loss of $\cdot(CH_2)_4CH_3$ from *m/z* 154].

*(ix) Incubation of 9(*S*)-HPOD*. Stirring 9(*S*)-HPOD (200 μM) at 23°C for 10 min with the whole homogenate preparation from potato stolons afforded compounds **1a**, **3a**, **7a**, and **9a** in a proportion closely similar to that observed following incubation of linoleic acid. The enzymes catalyzing hydroperoxide metabolism were mainly located in the particle fraction sedimenting at 105,000 × *g* as shown by experiments in which 9(*S*)-HPOD incubated with this fraction was converted into a mixture of **1a**, **3a**, **7a**, and **9a**. It seemed likely that an AOS was involved in the conversion of 9(*S*)-HPOD into α-ketol **7a** and cyclopentenone **3a**, and it was therefore of interest to study the metabolism of 9(*S*)-HPOD by another AOS. To this end, a suspension of the 105,000 × *g* particle fraction of corn seed homogenate was stirred with 9(*S*)-HPOD, and the methyl-esterified reaction product was analyzed by HPLC. As seen in Figure 3, the preparation of corn seed AOS produced a high yield of **7a** (analyzed as its methyl ester **7b**) but only a very small amount of cyclopentenone **3a** (analyzed as **3b**; ratio **3a**/**7a**, 0.005). The corresponding ratio observed with the AOS from potato stolons was 0.11 (Fig. 3).

*(x) Incubation of linolenic acid and 9(*S*)-HPOT*. As seen in Figure 1, linolenic acid (200 μM) was converted by the whole homogenate preparation of potato stolons in a way analogous to that of linoleic acid. In the methyl-esterified product, divinyl ether **2b** (4% of the product) and α-ketol **8b** (50%) were identified using the authentic reference compounds, whereas the structure of epoxy alcohol **10b** (31%) was tentatively assigned as methyl 10(*S*),11(*S*)-epoxy-9(*S*)-hydroxy-12(*Z*),15(*Z*)-octadecadienoate on the basis of analysis by GC–MS and on experiments in which isomeric trihydroxyesters (13) were isolated and characterized following mild acid-catalyzed conversion of **10b**. Compound **11b** (6%)

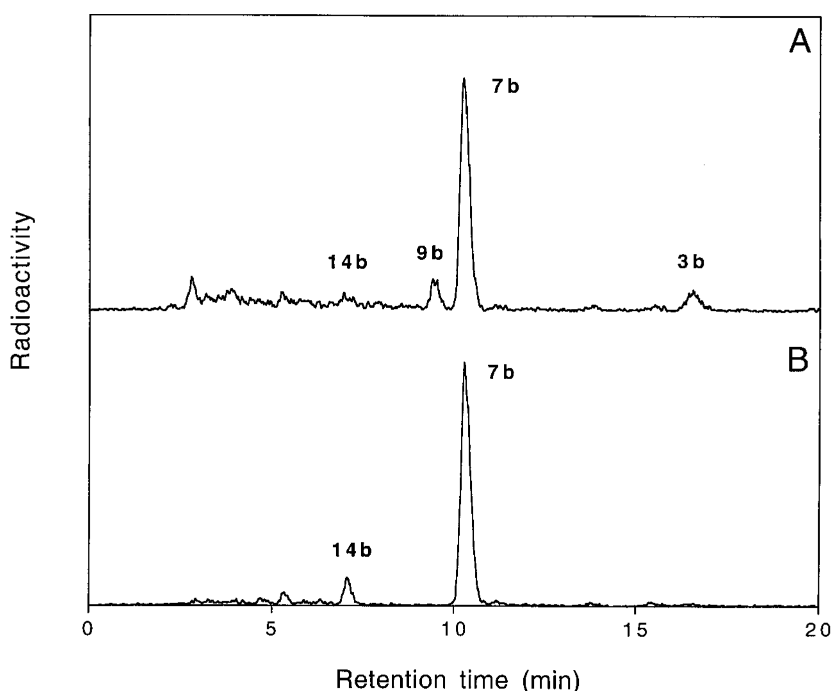


FIG. 3. Analysis by reversed-phase radio high-performance liquid chromatography of methyl-esterified reaction products obtained following incubations of [1-^{14}C]9(*S*)-hydroperoxy-10(*E*),12(*Z*)-octadecadienoic acid (100 μM) at 23°C for 2 min with the 105,000 × *g* particle fraction of homogenate of potato stolons (panel A) or with the corresponding fraction of homogenate of corn seed (panel B). The column was eluted at a flow rate of 1.5 mL/min with acetonitrile/water (65:35, vol/vol). The structures of the compounds indicated are given in Scheme 1. Compound **1b** was not eluted from the column during the time period used, and under the conditions used, compound **9b** suffered partial hydrolysis into more polar trihydroxy derivatives.

was a new cyclopentenone and was characterized as described below. A similar pattern of products was observed following incubation of 9(*S*)-HPOT.

*(xi) Structure of compound **11b***. The UV spectrum of **11b** showed a λ_{max} (EtOH) = 217 nm due to the cyclopentenone chromophore. Analysis by GC–MS showed a C-value of 20.11 and a mass spectrum exhibiting prominent ions at *m/z* 306 (1%; M^+), 275 (3; M^+ – 31; loss of $\cdot OCH_3$), 238 (4; M^+ – 68; loss of C_5H_8), 150 [9; M^+ – 156; β-cleavage with loss of CH_2=CH–$(CH_2)_5COOCH_3$], 121 (15), and 82 (100; C_5H_6O; loss of C_5H_8 from *m/z* 150). Treatment of **11b** with 0.1 M sodium hydroxide resulted in epimerization at C-9 and, following reesterification, to the formation of the side-chain *trans* derivative **12b**. As would be expected (12,19), the mass spectrum of **12b** was very similar to that of **11b**, but the C-value was lower (Table 1). Catalytic hydrogenation of **11b** afforded a tetrahydro derivative that was identical in all respects to **4b** prepared by hydrogenation of **3b**. The double bond in the pentenyl side chain of **11b** was localized by oxidative ozonolysis, which afforded tetramethyl decane-1,8,9,10-tetracarboxylate as the predominant product. Prominent ions in the mass spectrum of this compound were observed at *m/z* 343 (100%; M^+ – 31; loss of $\cdot OCH_3$), 310 (48; M^+ – 2 × 32); 229 [32; M^+ – 145; cleavage at C-8/C-9 and elimination of $\cdot CH(COOCH_3)$–CH_2COOCH_3], 218 [38; M^+ – 156; β-cleavage with loss of CH_2=CH–$(CH_2)_5COOCH_3$], 186 (70; *m/z* 218 – 32), 146 {39; cleavage at C-8/C-9 and charge retention in the fragment $[CH_3OOC–CH_2–CH=COH(OCH_3)]^+$}, 114 (43; *m/z* 146 – 32), and 55 (64).

Analysis of **11b** by CP-HPLC [solvent system, 2-propanol/hexane (2:8, vol/vol)] showed two peaks in a 1:1 ratio (7.5 and 10.0 mL), thus demonstrating that **11b** was a racemate due to equal parts of the 9(*R*),13(*R*) and 9(*S*),13(*S*) enantiomers. Based on the results mentioned, **11a** was assigned the structure (±)-2-oxo-5-[2′(*Z*)-pentenyl]-3-cyclopentene-1-octanoic acid.

Trapping experiments. (i) Incubations in the presence of GSH-px. Linoleic acid (200 μM) was stirred with a suspension of the 105,000 × *g* particle fraction of homogenate of potato stolons in the presence of GSH-px (2 U/mL) and GSH (2 mM). Analysis of the methyl-esterified product by SP-HPLC showed a major peak accounting for 90% of the total radioactivity. This material cochromatographed with the methyl ester of 9-HOD and was conclusively identified as this compound by analysis of the Me_3Si derivative by GC–MS. Only trace amounts (<1%) of the regioisomeric 13-HOD methyl ester could be detected. Analysis of the 9-HOD methyl ester by CP-HPLC revealed it to be the natural *S* enantiomer.

(ii) Trapping of an allene oxide with methanol. 9(*S*)-HPOD (63 μM) was stirred at 0°C for 30 s with 2 mL of a suspen-

sion of the 105,000 × g particle fraction of homogenate of potato stolons. Methanol (40 mL) was added, and the mixture was kept at 23°C for 1 h. Analysis of the reaction product by SP-HPLC [solvent system, 2-propanol/hexane/acetic acid (1:99:0.01, by vol)] revealed the presence of compound **13a** (16% of the total radioactivity; 9.2 mL effluent)) in addition to α-ketol **7a** (51%; 24.8 mL) and other minor compounds. Formation of **13a** successively decreased when longer times of incubations were used and was not noticeable at 5 min of incubation. Its identity with 9-methoxy-10-oxo-12(*Z*)-octadecenoic acid was ascertained using GC–MS analysis of the methyl ester with the authentic compound as reference. The mass spectrum of **13b** showed prominent ions at *m/z* 340 (2%; M^+), 309 (5; M^+ – 31; loss of $\cdot OCH_3$), 201 [100; $CH_3O^+=CH–(CH_2)_7COOCH_3$], 169 (24; *m/z* 201 – 32), 137 (44; *m/z* 201-2 × 32), and 71 (35). Analysis of **13b** by CP-HPLC [solvent system, 2-propanol/hexane (4:96, vol/vol)] showed separation into two enantiomers, tentatively assigned as methyl 9(*R*)-methoxy-10-oxo-12(*Z*)-octadecenoate (76%; 7.5 mL effluent) and methyl 9(*S*)-methoxy-10-oxo-12(*Z*)-octadecenoate (24%; 6.5 mL). Steric analysis of **13b** obtained as a trapping product in the corn seed AOS-catalyzed reaction gave a similar enantiomeric composition, i.e., 73:27.

As shown in previous work, α-methoxy ketones are formed as trapping products of unstable allene oxides (8,9), and the transient appearance of **13a** indicated the formation of 9(*S*),10-epoxy-10,12(*Z*)-octadecadienoic acid from 9(*S*)-HPOD catalyzed by potato stolon AOS. The half-life of the allene oxide estimated from the rate of disappearance of **13a** was 44 s (0°C; pH 7.4). The half-life of the corresponding allene oxide generated in the corn seed AOS system was 33 s, a value almost identical to that (34 s) previously published (9).

(iii) Intramolecular trapping of an allene oxide—formation of macrolactones. 9(*S*)-HPOD (100 μM) was stirred at 0°C for 30 s with 11 mL of a suspension of the 105,000 × g particle fraction of homogenate of potato stolons. Ice-cold acetonitrile (220 mL) was added, and the mixture was kept at 0°C for 1 h. The mixture was extracted with hexane (no acidification), and the product was subjected to SP-HPLC using a solvent system of 2-propanol/hexane (0.2:99.8, vol/vol). Two peaks of nonpolar radioactive materials appeared, i.e., **16** (3.9 mL effluent; 25%) and **15** (6.2 mL; 75%). The mass spectrum of **16** showed prominent ions at *m/z* 294 (13%; M^+), 223 [4; M^+ – 71; loss of $\cdot(CH_2)_4CH_3$], 169 (13), 98 (100), and 55 (63), whereas the mass spectrum of **15** exhibited ions at *m/z* 294 (2%; M^+), 183 [10; M^+ – 111; loss of $\cdot CH_2–CH=CH–(CH_2)_4CH_3$], 155 [100; M^+ – 139; loss of $\cdot CO–CH_2–CH=CH–(CH_2)_4CH_3$], 109 (38), and 55 (39). Authentic macrolactones **15** and **16**, which were prepared by short-time incubation of 9(*S*)-HPOD with corn seed AOS (9), gave identical analytical results. Interestingly, a significant difference between the proportions of **15**/**16** was noted, i.e., 75:25 using the potato stolon AOS and 89:11 using the corn seed AOS.

*Formation of **3a** and **14a** under various conditions. (i) Effect of pH, temperature, and substrate concentration*. In a series of incubations, 9(*S*)-HPOD was stirred with the 105,000 × g particle fraction from homogenate of potato stolons or with the corresponding fraction from corn seed homogenate. The compositions of the reaction products were determined by SP-radio-HPLC. As seen in Table 3, formation of **3a** (relative to **7a**) was temperature- and pH-dependent. On the other hand, diluting the enzyme preparation 10-fold, or changing the substrate concentration in the range 30–300 μM, did not alter the ratio **3a**/**7a**.

γ-Ketols are significant products in incubations with corn seed AOS (8,15). Although the γ-ketol **14a** accounted for only 2% of the recovered radioactivity in the potato stolon incubations, it appeared to be of interest to include this AOS product in the analyses and to study the pH-dependency of its formation. γ-Ketols can give a by-product when methyl-esterified by diazomethane (21), and the radio-HPLC analysis was accordingly carried out on the nonesterified incubation products. As seen in Table 3, formation of **14a** showed a pH-dependency in agreement with its postulated formation from the protonated allene oxide (22). The reason for the lower ratio

TABLE 3
Formation of Allene Oxide Synthase Products Under Various Conditions

	Parameter changed[a]						**3a**	**7a**
Tissue	Concentration	Temperature	pH	Time	**3a/7a**	**14a/7a**	Enantiomers, *S*/*R*	
Potato	—	—	—	—	0.11	0.04	50:50	9:91
Potato	—	0°C	—	5 min	0.03	—	50:50	6:94
Potato	—	37°C	—	—	0.19	—	49:51	11:89
Potato	—	—	6.7	—	0.08	0.08	—	—
Potato	—	—	8.0	—	0.14	0.02	—	—
Potato	30 μM	—	—	—	0.11	—	49:51	9:91
Potato	300 μM	—	—	—	0.11	—	50:50	10:90
Potato, 1:10	—	—	—	—	0.11	—	50:50	10:90
Corn	—	—	—	—	0.005	0.11	—	34:66
Corn	—	0°C	—	5 min	<0.005	—	—	26:74

[a]Standard conditions: 9(*S*)-Hydroperoxy-10(*E*),12(*Z*)-octadecadienoic acid (100 μM) was stirred for 2 min at 23°C with a suspension of the 105,000 × g particle fraction of homogenate of potato stolons, pH 7.4. Abbreviations: **3a**, 2-oxo-5-pentyl-3-cyclopentene-1-octanoic acid (1,5-*cis* isomer); **7a**, 9-hydroxy-10-oxo-12(*Z*)-octadecenoic acid; **14a**, 13-hydroxy-10-oxo-11(*E*)-octadecenoic acid .

TABLE 4
Formation of Compound 3a in Homogenates of Tissues from the Potato Plant[a]

	Compound **3a** (nmol/g)	
Tissue	Linoleic acid incubation	9(*S*)-HPOD incubation
Stolons	79.4 ± 9.5	84.3 ± 6.4
Tubers, 5–10 mm diam.	20.7 ± 7.1	24.7 ± 4.3
Tubers, 30–40 mm diam.	7.4 ± 2.8	7.7 ± 2.8
Leaves	<1.5	<1.5
Roots	159.0 ± 21.8	172.8 ± 15.5

[a]For determination of **3a**, 2 g of tissue was minced and homogenized in 20 mL of 0.1 M potassium phosphate buffer pH 7.4. Five milliliters of the filtrate were prewarmed at 23°C and subsequently stirred with 200 μM linoleic acid or 200 μM 9(*S*)-HPOD for 10 min. The amounts of **3a** were determined by gas chromatography–mass spectrometry using 12-oxostearic acid as internal standard. For abbreviation see Table 3.

14a/**7a** in the potato stolon incubation compared to the corn seed incubation is unknown.

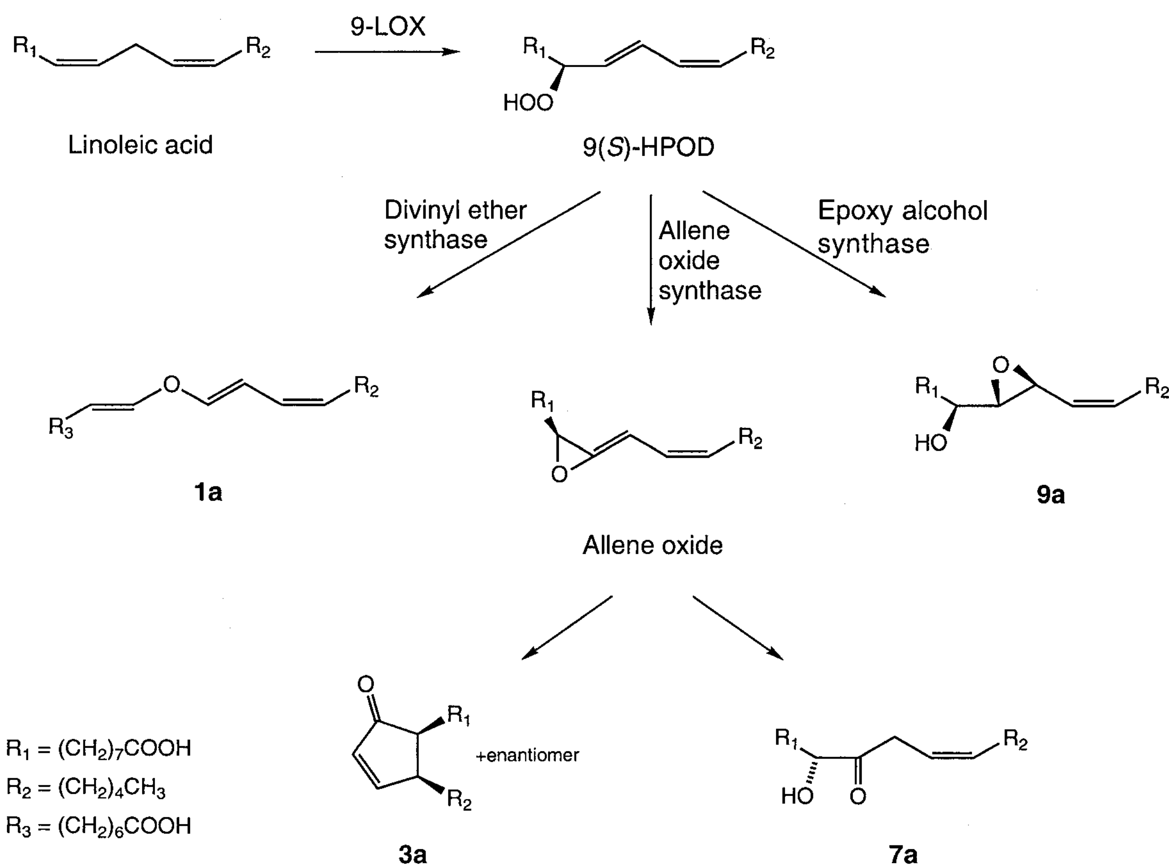
(*ii*) *Stereochemical compositions of **3a** and **7a***. Compounds **3b** and **7b** obtained from the various incubations shown in Table 3 were subjected to steric analysis using CP-HPLC. As seen in Table 3, cyclopentenone **3a** was consistently formed as a racemate, whereas the α-ketol **7a** was enriched with respect to the *R* enantiomer. The latter result was in agreement with its mode of formation from the allene oxide partly by S_N2 type of hydrolysis at C-9 (8,9,22,23). Interestingly, **7a** generated in the potato stolon system had an enantiomeric purity that was significantly higher than that of **7a** produced in the corn seed system (Table 3).

(*iii*) *Formation of **3a** in different parts of the potato plant*. Whole homogenate preparations (1:10, wt/vol; 5 mL) of different parts of the potato plant were stirred with linoleic acid or 9(*S*)-HPOD (200 μM), and the amounts of **3a** were determined by GC–MS. As seen in Table 4, the highest levels were obtained with homogenates of roots and stolons. Homogenate of potato tubers gave smaller amounts, and leaves produced no detectable levels of **3a**. The fact that multiple enzymes metabolizing 9(*S*)-HPOD were present in the homogenates used meant that the amounts of **3a** did not necessarily reflect the capacity of formation of **3a**. Rather, the values indicated the partitioning of 9(*S*)-HPOD between different hydroperoxide-degrading enzymes present in the tissue preparations.

DISCUSSION

The present study is mainly concerned with the metabolism of linoleic acid by an enzyme preparation from potato stolons. The initial reaction was catalyzed by a 9-lipoxygenase (9-LOX) and resulted in the formation of 9(*S*)-HPOD. Three enzymes, i.e., divinyl ether synthase, epoxy alcohol synthase and AOS, catalyzed the further metabolism of 9(*S*)-HPOD to afford colneleic acid (**1a**), the epoxy alcohol 10(*S*),11(*S*)-epoxy-9(*S*)-hydroxy-12(*Z*)-octadecenoic acid (**9a**), and the allene oxide 9(*S*),10-epoxy-10,12(*Z*)-octadecadienoic acid, respectively (Scheme 2). Of these compounds, colneleic acid was originally obtained following incubation of linoleic acid with a preparation of potato tubers (14), and the epoxy alcohol was recently isolated as one of the major products of linoleic acid metabolism in homogenate of potato leaves (13). The allene oxide, like allene oxides derived from the 13-hydroperoxides of linoleic and linolenic acids (8,23), was highly unstable and had an estimated half-life at 0°C of 44 s. No attempts at its isolation were made; however, its presence was proved by trapping experiments with methanol, which afforded 9-methoxy-10-oxo-12(*Z*)-octadecenoic acid. Furthermore, when brief incubations of 9(*S*)-HPOD were carried out at 0°C and interrupted by the addition of 20 vol of the nonhydroxylic solvent acetonitrile, the two macrolactones **15** and **16** were formed. These compounds had been encountered in an earlier study as products formed by intramolecular trapping of the allene oxide structure (9). Under normal conditions for incubation, further conversion of the allene oxide resulted in the formation of compounds **7a** and **3a** (Scheme 2). α-Ketol **7a** was originally isolated and characterized in a study of linoleic acid metabolism in corn seed homogenate (24) and was later shown to be a product formed by hydrolysis of an allene oxide generated by corn seed AOS (9). Cyclopentenone **3a**, a new oxylipin, was fully characterized by chemical and spectral methods. Steric analysis by NMR spectroscopy, CP-HPLC, and GLC demonstrated that the two appendages of the five-membered ring had the *cis* relative configuration and that the compound was a racemate consisting of equal parts of the 9(*R*),13(*R*) and 9(*S*),13(*S*) enantiomers. Compounds related to **3a** had been encountered previously in studies of the formation of cyclopentenones employing the hydroperoxides 9(*S*)-hydroperoxy-6(*Z*),10(*E*),12(*Z*)-octadecatrienoic acid (5,25) and 9(*R,S*)-hydroperoxy-10(*E*),12(*E*)-octadecadienoic acid (26), i.e., two hydroperoxides which are not typical constituents of plant tissue. In the present study, natural linoleic acid 9-hydroperoxide served as the precursor of cyclopentenone, and this result was extended to linolenic acid and its 9-hydroperoxide, which were metabolized to cyclopentenone **11a**. This compound [10-oxo-11,15(*Z*)-phytodienoic acid] was a regioisomer of the jasmonate precursor 12-oxo-10,15(*Z*)-phytodienoic acid, differing from this compound with respect to the positions of the keto group and the double bond in the five-membered ring.

Studies of the mode of formation of cyclopentenone **3a** and α-ketol **7a** were carried out by performing incubations of 9(*S*)-HPOD with the 105,000 × *g* particle fraction of homogenate of potato stolons. As seen in Table 3, formation of **3a**, as monitored by the ratio **3a**/**7a**, was temperature- and pH-dependent. Interestingly, when different concentrations of 9(*S*)-HPOD (30–300 μM) were used, thus providing varying concentrations of the corresponding allene oxide, the ratio **3a**/**7a** remained unchanged. Likewise, diluting the enzyme preparation 10-fold did not affect the ratio. These results, and the fact that **3a** was isolated as a racemate, suggested that **3a** (and **11a**) were formed from their allene oxide precursors by way of nonenzymatic cyclization. This conclusion was unexpected in view of the fact that earlier reported cases of nonen-



SCHEME 2

zymatic cyclization of fatty acid allene oxides had been restricted to allene oxides possessing a double bond in the β,γ-position relative to the epoxide group (5,15,23,27). In agreement with this notion, it was confirmed in the present study that the allene oxide generated from 9(*S*)-HPOD using the previously studied AOS from corn seeds (8,9,15) was a very poor precursor of cyclopentenone (Table 3, Fig. 3). Interestingly, the potato stolon and corn seed AOS-catalyzed reactions differed also in another way, i.e., with respect to the enantiomeric composition of the α-ketol product (Table 3). The large proportion of the *R* enantiomer in **7a** produced by the potato stolon AOS indicated that this α-ketol was formed from its allene oxide precursor largely by an S_N2-type of hydrolysis (*cf.* Refs. 8,23). A third difference between the potato stolon and corn seed AOS preparations was the different extent of formation of the γ-ketol **14a** (Table 3). Further studies are needed in order to explain these results. It can only be speculated that a configurational or conformational property in the allene oxide generated by the potato stolon AOS exists, or is induced, which has consequences for the further conversion of the allene oxide.

It seems likely that the cyclopentenones **3a** and **11a** can be further converted in plant tissue in the same way as 12-oxo-PDA, i.e., by reduction of the ring double bond and β-oxidation. Such transformations as well as the biological properties of the new cyclopentenones are being studied (Kod, Y., and Hambeg, M., unpublished data).

ACKNOWLEDGMENTS

Gunvor Hamberg is thanked for expert technical assistance. The author is indebted to Prof. Per-Erik Jansson and Reine Eserstam (Clinical Research Center, Huddinge University Hospital, Stockholm) for their help with the NMR analyses and to Dr. A.N. Grechkin (Institute of Biochemistry and Biophysics, Russian Academy of Sciences, Kazan, Russia) and Prof. B.A. Hess (Department of Chemistry, Vanderbilt University, Nashville, TN) for discussion of possible mechanisms of allene oxide cyclization into cyclopentenones. This work was supported by the Swedish Medical Research Council (Proj. No. 03X-5170) and by Vesical Co. (Stockholm).

REFERENCES

1. Creelman, R.A., and Mullet, J.E. (1997) Biosynthesis and Action of Jasmonates in Plants, *Annu. Rev. Plant Physiol. Plant Mol. Biol. 48*, 355–381.
2. Vick, B.A., and Zimmerman, D.C. (1983) The Biosynthesis of Jasmonic Acid: A Physiological Role for Plant Lipoxygenase, *Biochem. Biophys. Res. Commun. 111*, 470–477.
3. Mueller, M.J. (1997) Enzymes Involved in Jasmonic Acid Biosynthesis, *Physiol. Plant. 100*, 653–663.
4. Grechkin, A. (1998) Recent Developments in Biochemistry of the Plant Lipoxygenase Pathway, *Prog. Lipid Res. 37*, 317–352.

5. Ziegler, J., Wasternack, C., and Hamberg, M. (1999) On the Specificity of Allene Oxide Cyclase, *Lipids 34*, 1005–1015.
6. Weber, H., Vick, B.A., and Farmer, E.E. (1997) Dinor-oxo-phytodienoic Acid: A New Hexadecanoid Signal in the Jasmonate Family. *Proc. Natl. Acad. Sci. USA 94*, 10473–10478.
7. Matthew, J.A., Chan, H.W.-S., and Galliard, T. (1977) A Simple Method for the Preparation of Pure 9-D-Hydroperoxide of Linoleic Acid and Methyl Linoleate Based on the Positional Specificity of Lipoxygenase in Tomato Fruit, *Lipids 12*, 324–326.
8. Hamberg, M. (1987) Mechanism of Corn Hydroperoxide Isomerase: Detection of 12,13(*S*)-Oxido-9(*Z*),11-octadecadienoic Acid, *Biochim. Biophys. Acta 920*, 76–84.
9. Hamberg, M., and Hughes, M.A. (1988) Biosynthesis and Conversions of Fatty Acid Allene Oxides, in *Advances in Prostaglandin, Thromboxane, and Leukotriene Research* (Samuelsson, B., Wong, P.Y.-K., and Sun, F.F., eds.), Vol. 19, pp. 64–69, Raven Press, New York.
10. Hamberg, M. (1988) Fatty Acid Allene Oxides II. Formation of Two Macrolactones from 12,13(*S*)-Epoxy-9(*Z*),11-octadecadienoic Acid, *Chem. Phys. Lipids 46*, 235–243.
11. Hamberg, M., Miersch, O., and Sembdner, G. (1988) Absolute Configuration of 12-Oxo-10,15(*Z*)-phytodienoic Acid, *Lipids 23*, 521–524.
12. Hamberg, M., and Hughes, M.A. (1988) Fatty Acid Allene Oxides. III. Albumin-Induced Cyclization of 12,13(*S*)-Epoxy-9(*Z*),11-octadecadienoic Acid, *Lipids 23*, 469–475.
13. Hamberg, M. (1999) An Epoxy Alcohol Synthase Pathway in Higher Plants: Biosynthesis of Antifungal Trihydroxy Oxylipins in Leaves of Potato, *Lipids 34*, 1131–1142.
14. Galliard, T., and Phillips, D.R. (1972) The Enzymic 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.
15. Hamberg, M., and Fahlstadius, P. (1990) Allene Oxide Cyclase: A New Enzyme in Plant Lipid Metabolism, *Arch. Biochem. Biophys. 276*, 518–526.
16. Hamberg, M. (1998) A Pathway for Biosynthesis of Divinyl Ether Fatty Acids in Green Leaves, *Lipids 33*, 1061–1071.
17. Baertschi, S.W., Ingram, C.D., Harris, T.M., and Brash, A.R. (1988) Absolute Configuration of *cis*-12-Oxophytodienoic Acid of Flaxseed: Implications for the Mechanism of Biosynthesis from the 13(*S*)-Hydroperoxide of Linolenic Acid, *Biochemistry 27*, 18–24.
18. Zimmerman, D.C., and Feng, P. (1978) Characterization of a Prostaglandin-Like Metabolite of Linolenic Acid Produced by a Flaxseed Extract, *Lipids 13*, 313–316.
19. Vick, B.A., Zimmerman, D.C., and Weisleder, D. (1979) Thermal Alteration of a Cyclic Fatty Acid Produced by a Flaxseed Extract, *Lipids 14*, 734–740.
20. Anteunis, M., and Danneels, D. (1975) Stereochemical Aspects of Proton Chemical Shifts III. Configurational Assignments in Pentacyclic Systems Without Recourse to a Karplus Equation, *Org. Magn. Reson. 7*, 345–348.
21. Abian, J., Gelpi, E., and Pages, M. (1991) Effect of Abscisic Acid on the Linoleic Acid Metabolism in Developing Maize Embryos, *Plant Physiol. 95*, 1277–1283.
22. Grechkin, A.N., Kuramshin, R.A., Safonova, E.Y., Latypov, S.K., and Ilyasov, A.V. (1991) Formation of Ketols from Linolenic Acid 13-Hydroperoxide *via* Allene Oxide. Evidence for Two Distinct Mechanisms of Allene Oxide Hydrolysis, *Biochim. Biophys. Acta 1086*, 317–325.
23. Brash, A.R., Baertschi, S.W., Ingram, C.D., and Harris, T.M. (1988) Isolation and Characterization of Natural Allene Oxides: Unstable Intermediates in the Metabolism of Lipid Hydroperoxides, *Proc. Natl. Acad. Sci. USA 85*, 3382–3386.
24. Gardner, H.W. (1970) Sequential Enzymes of Linoleic Acid Oxidation in Corn Germ: Lipoxygenase and Linoleate Hydroperoxide Isomerase, *J. Lipid Res. 11*, 311–321.
25. Vick, B.A., Feng, P., and Zimmerman, D.C. (1980) Formation of 12-[^{18}O]Oxo-*cis*-10,*cis*-15-Phytodienoic Acid from 13-[^{18}O]-Hydroperoxylinolenic Acid by Hydroperoxide Cyclase, *Lipids 15*, 468–471.
26. Grechkin, A.N., and Hamberg, M. (1999) Formation of Cyclopentenones from All-(*E*) Hydroperoxides of Linoleic Acid *via* Allene Oxides. New Insight into the Mechanism of Cyclization, *FEBS Lett. 466*, 63–66.
27. Grechkin, A.N. (1994) Cyclization of Natural Allene Oxide Fatty Acids. The Anchimeric Assistance of β,γ-Double Bond Beside the Oxirane and the Reaction Mechanism, *Biochim. Biophys. Acta 1213*, 199–206.

[Received January 6, 2000, and in revised form February 18, 2000; revision accepted March 1, 2000]