hydroxy-(2E)-nonenal is formed by protein homogenates of soya bean cotyledons [3]. However, until now it is still under discussion as to whether these aldehydes are formed in plants exclusively by the action of enzymes [3] or whether they are at least partially the result of autoxidative processes, i.e. originating from the corresponding (3Z)-aldehydes.

To address this question, we incubated (3Z)-hexenal (Figure 1) or (3Z)-nonenal (results not shown) with the recombinant barley LOX-100 [4]. This LOX form is induced in jasmonate-treated leaves and is located together with hydroperoxide lyase in chloroplasts of barley leaves [5]. Furthermore, we incubated (3Z)-hexenal or (3Z)-nonenal with oxidizing chemicals, such as the corresponding fatty acid hydroperoxides already detected in barley leaves under the same conditions, or with H2O2 or with both. For both aldehydes similar results were observed. As shown for HHE formation (Figure 1), no differences were found when (3Z)-hexenal was incubated either alone with (13S,9Z,11E)-13-hydroperoxy-9,11-octadecadienoic acid (13-HPOD)/T or in combination with LOX-100. In contrast to earlier findings, these results may suggest that the formation of 4-hydroxy-(2E)-alkenals in plants may be due to autoxidation and not the conversion of these aldehydes by LOXs, as suggested before [3].

References

Received 23 June 2000

Lipoxygenase pathway in tulip: biosynthesis of ketols
A. N. Grechkin*, L. S. Mukhtarova* and M. Hamberg†

*Kazan Institute of Biochemistry and Biophysics, Russian Academy of Sciences, P.O. Box 30, Kazan, 420503, Russia, and †Department of Physiological Chemistry II, Karolinska Institutet, S-17177, Stockholm, Sweden

Abstract
The metabolism in vitro of [1-14C]linoleate, [1-14C]linolenate and their 9(S)-hydroperoxides in tulip (Tulipa gesneriana) was found to be under the control of 9-lipoxygenase and allene oxide synthase, and directed towards α-ketol, γ-ketol and the novel compound (12Z)-10-oxo-11-hydroxy-12-octadecadienoic acid (10,11-ketol). Potent activity of allene oxide cyclase (in bulbs) and a new enzyme, γ-ketol reductase (in bulbs and leaves), was detected. Metabolism in flowers is directed predominantly towards α-ketol hydroperoxide.

Introduction
Allene oxide synthase (AOS) is one of the most important enzymes of the plant lipoxygenase (LOX) pathway. Allene oxides, short-living products of AOS, undergo spontaneous hydrolysis into ketols [1–4]. An allene oxide formed from α-linolenate 13-hydroperoxide (13-HPOT) forms cyclopentenone as a predominant product in the presence of allene oxide cyclase (AOC) [2]. The product of its cyclization, (15Z)-12-oxo-10,15-phytodienoic acid (12-oxo-PDA), is a precursor of a phytohormone 7-iso-jasmonic acid [5]. The physiological significance of ketols, the major AOS products, remain poorly understood. The present paper is concerned with detection of the ‘ketol route’ of AOS tissues possessing 9-LOX and AOS activities, is entirely directed towards ketols and related products.
Experimental

Extracts of tulip bulbs, leaves and flower petals were incubated with [1-14C]linoleate, [1-14C]α-linolenate, (10E,12Z)-9-hydroxyperoxy-10,12-octadecadienoic acid ([1-14C]9-HPOD) or (10E,12Z,15Z)-[1-14C]9-hydroperoxy-10,12,15-octadecatrienoic acid ([1-14C]9-HPOT). Fractions obtained by differential centrifugation (105 000 g) of bulb homogenate were also incubated with 13-HPOT. Protein fraction from the supernatant of tulip-leaf homogenate was precipitated with (NH₄)SO₄ (70 %-satd). After centrifugation at 9300 g for 16 min, the resulting pellet was stored in a freezer. Parts of dissolved enzyme were incubated with the following five substrates: (1) [1-14C]y-ketol, (11E)-10-oxo-13-hydroxy-11-octadecenoic acid; (2) [1-14C]hydro(pero)xy α-ketol; (3) [1-14C]12-0x0-PDA; (4) [1-14C](10E,12Z)-9-oxo-10,12-octadecadienoic acid; and (5) [1-14C](9Z,11E)-13-oxo-9,11-octadecadienoic acid. Extraction and radio-HPLC and GC-MS analyses of products were performed as described previously [3,4].

Results

Metabolism of [1-14C]linoleic acid and [1-14C]9(S)-HPOD in tulip bulbs, leaves and flowers

Two main products, (12Z)-9-hydroxy-10-oxo-12-octadecenoic acid (α-ketol) and (11E)-10-oxo-13-hydroxy-11-octadecenoic acid (γ-ketol), and a minor metabolite, (12Z)-10-oxo-11-hydroxy-12-octadecenoic acid (10,11-ketol), were formed after a 15 min incubation of [1-14C]linoleate or [1-14C]9-HPOD with tulip-bulb extract. Prolonged (60 min) incubations were accompanied by a fall in the radioactivity of the product (γ-ketol), and an increase of label incorporation into α-ketol hydroperoxide. Similar patterns of the same metabolites were found after incubations of [1-14C]linoleate with leaf extract. If incubations with bulb and leaf preparations afforded mostly α-ketol, during the incubations with flower extracts, label was incorporated mainly into α-ketol hydroperoxide.

Metabolism of [1-14C]α-linolenic acid and its hydroperoxides

Exogenous [1-14C]α-linolenic acid was also rapidly converted into products during the incubation with tulip leaf and bulb extracts. The main metabolites were identified as (12Z,15Z)-9-hydroxy-10-oxo-12,15-octadecadienoic acid (α-ketol), (11E,15Z)-10-oxo-13-hydroxy-10,15-octadecadienoic acid (γ-ketol) and (11E,15Z)-9-hydroxy-10-oxo-13-hydro(pero)xy-11,15-octadecadienoic acid. The same metabolites were detected after the incubation of leaf extract with [1-14C]9(S)-HPOT.

Incubations of [1-14C]13-HPOT with filtrate of bulb homogenate led to two products, (9Z,15Z)-12-oxo-13-hydroxy-9,15-octadecadienoic acid (α-ketol) and (15Z)-12-oxo-10,15-phytodienoic acid in a ratio of 1:12. The resulting 12-oxo-PDA was represented by 99.5 % optically pure (9S,13S) epimer. Thus the results demonstrate that tulip bulbs exhibit high activity of AOC. Incubations of 13-HPOT with sub-cellular fractions revealed that the main part of enzyme activity is associated with microsomes.
Discussion
The results of the present work demonstrate that the LOX pathway in tulip bulbs and leaves operates via the 9-LOX and AOS activities. Tulip is the second plant species known to possess predominantly 9-LOX activity in its leaves, along with recently characterized potato leaves [6].

It seems logical to assume that ketols, being the predominant LOX products in such tissues as tulip bulbs and maize seeds, must have their own physiological importance. As found recently, the y-ketol (11E)-10-oxo-13-hydroxy-10-octadecenoic acid and a related macrolactone, (11E)-10-oxo-11-octadecen-13-olide, isolated from maize seeds, exhibit strong cytostatic activity [1]. One might propose, in connection with this, that reduction of y-ketols and related oxylipins by y-ketol reductase may have physiological importance as an intracellular mechanism for detoxification of these metabolites. As reported recently [7], (12Z,15Z)-9-hydroxy-10-oxo-12,15-octadecadienoic acid (y-ketol) is an endogenous flowering factor in Lemna paucicostata.

This work was supported by grant 00-04-48221 from the Russian Foundation of Basic Research and grant 00-15-97904 (program for the support of leading scientific schools).

References

Received 7 August 2000

Potato tubers exhibit both homolytic and heterolytic hydroperoxide fatty acid-cleaving activities
M.-L. Fauconnier*1, J. Delcarte*, P. Hoyaux*, P. du Jardin† and M. Marlier*
*Unité de Chimie Générale et Organique, Faculté Universitaire des Sciences Agronomiques de Gembloux, Passage des Déportés, 2-830 Gembloux, Belgium, and †Unité de Biologie Végétale, Faculté Universitaire des Sciences Agronomiques de Gembloux, Passage des Déportés, 2-8530 Gembloux, Belgium

Abstract
The action of a crude potato-tuber extract on 9- and 13-hydroperoxides of linoleic and linolenic acids was investigated. HPLC analysis revealed that 50% of the 9-hydroperoxide isomers and almost all the 13-hydroperoxide isomers were rapidly enzymically metabolized. No degradation of fatty acid hydroperoxides was observed with a thermally denatured enzymic extract. GC-MS identification of the volatiles formed by the reaction revealed that no volatiles were detected from the 9-hydroperoxide isomers, whereas 13-hydroperoxide of linolenic acid was cleaved into (Z)-3-hexenal, pentenols or dimers of pentene.

Key words: fatty acid hydroperoxide, hydroperoxide lyase, lipoxigenase, Solanum tuberosum L.
†To whom correspondence should be addressed (e-mail fauconnier.ml@fsagx.ac.be).

Introduction
Depending on botanical origin and on reaction conditions, plant lipoxigenases can form variable amounts of 13- or 9-hydroperoxides of linoleic or linolenic acid. Fatty acid hydroperoxides can be degraded further in a variety of compounds implicated in essential physiological roles in plants (jasmonic acid, traumatin) or responsible for the characteristic green note odour of plants and fruits (C9 or C15 aldehydes and alcohols) [1].

Four main fatty acid hydroperoxide-decomposing activities are described: allene oxide synthase catalyses the conversion of fatty acid hydroperoxides into allene oxides, precursors of y- and y-ketols and of jasmonic acid (from 13-hydroperoxide of linolenic acid) [2]. Peroxigenase and epoxigenase cause epoxidation of fatty acid hydroperoxides [3,4]. Divinyl ether synthase transforms fatty acid hydroperoxides into fatty acid divinyl ethers [5,6]. Finally, hydroperoxide lyase cleaves fatty acid hydroperoxides into alde-