Available online at www.sciencedirect.com
SCIENCE DIRECT®
Phytochemistry 66 (2005) 305–311
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Biosynthesis of mono- and sesquiterpenes in carrot roots and leaves (*Daucus carota* L.): metabolic cross talk of cytosolic mevalonate and plastidial methylerythritol phosphate pathways

Daniela Hampel [a], Armin Mosandl [a], Matthias Wüst [b,*]

[a] *Institut für Lebensmittelchemie, Johann Wolfgang Goethe-Universität, Marie-Curie-Strasse 9, D-60439 Frankfurt/Main, Germany*
[b] *Department of Life Technologies, University of Applied Sciences Valais, Route du Rawyl 47, CH-1950 Sion, Switzerland*

Received 13 August 2004; received in revised form 7 December 2004

Abstract

The biosynthesis of the monoterpenes terpinolene and myrcene and the sesquiterpene β-caryophyllene in roots and leaves of two carrot varieties (*Daucus carota* L. cultivars Bolero and Kazan) were investigated by in vivo feeding experiments with [5,5-$^2H_2$]-mevalonic acid lactone (d_2-MVL) and [5,5-$^2H_2$]-1-deoxy-D-xylulose (d_2-DOX). The volatiles of the tissues were extracted by stir bar sorptive extraction and analyzed using thermal desorption–multidimensional gas chromatography–mass spectrometry. The experiments demonstrate independent de novo-biosynthesis of terpenoids in carrot roots and in carrot leaves. In both plant tissues monoterpenes are biosynthesized exclusively via the 1-deoxy-D-xylulose/2-C-methyl-D-erythritol-4-phosphate (DOXP/MEP) pathway, whereas sesquiterpenes are generated by the classical mevalonic acid pathway as well as by the DOXP/MEP route. A more detailed investigation of carrot root tissues revealed that the biosynthesis of terpenes is mainly localized in the phloem. Nevertheless, in xylem a de novo-biosynthesis of terpenes was detectable as well, even in the absence of oil ducts in this tissue.

Keywords: *Daucus carota* L.; Umbelliferae; 1-Deoxy-D-xylulose; Mevalonic acid; Stir bar sorptive extraction; Thermal desorption–multidimensional gas chromatography–mass spectrometry

1. Introduction

Volatile terpenoids are mainly responsible for the typical aroma and flavour of carrots (*Daucus carota* L.). Taken together, mono- and sesquiterpenes represent approximately 98% of the volatile compounds (Alasalvar et al., 1998; Kjeldsen et al., 2001). Monoterpenes like sabinene, β-myrcene and *p*-cymene seem to be important contributors to the "carrot top" aroma with relatively high odour activity values (OAVs) (Kjeldsen et al., 2003). Sesquiterpenes like β-caryophyllene and α-humulene contribute to the "spicy" and "woody" notes, whereas the "sweet" note is mainly due to the norisoprenoid compound β-ionone. A detailed analysis of the terpene profile in foliage and roots revealed distinct differences in total amount and proportions of individual compounds and suggest that terpenoid metabolism differs substantially in these tissues (Selanik and Simon, 1987; Habegger and Schnitzler, 2000). Higher concentration of terpenoids in the root phloem than in the xylem has been explained by the observation that oil ducts, a possible site of volatile terpenoid biosynthesis, are found only in the phloem (Selanik and Simon, 1986). Between their number and the terpenoid level there is a positive correlation. Whether the terpenoids found in the xylem are translocated or synthesized there is

* Corresponding author. Tel.: +41 27 606 86 54; fax: +41 27 606 85 15.
E-mail address: matthias.wuest@hevs.ch (M. Wüst).

0031-9422/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2004.12.010

presently unknown. Despite their importance for the carrot flavour only little information is available on the biochemistry of terpene biosynthesis in carrots. Cyclization of neryl diphosphate to α-terpineol and isomerization of geraniol and geranyl phosphate to nerol and neryl phosphate, respectively, by cell-free enzyme systems from carrot leaves have previously been reported (Croteau et al., 1973; Shine and Loomis, 1974). However, labelling studies with carrot roots have not been performed up to now. Additionally, the biosynthetic origin of the building blocks of all terpenes, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), has not been investigated yet in carrots. Recently a mevalonate independent route for the biosynthesis of IPP and DMAPP has been discovered in bacteria, algae and higher plants, the novel 1-deoxy-D-xylulose/2-*C*-methyl-D-erythritol-4-phosphate (DOXP/MEP) pathway, which is unlike the classical cytosolic mevalonic acid (MVA) route localized in the plastids (Lichtenthaler et al., 1997a,b, 1999; Rohmer, 1999; Eisenreich et al., 2001, 2004). Monoterpenes are formed via the DOXP/MEP pathway, whereas sesquiterpenes are biosynthesized via the classical MVA route. However, there is no absolute compartmental separation of the two pathways and the extent of this cross-talk depends on the species and the physiological conditions (Luan and Wüst, 2002; Hemmerlin et al., 2003). Furthermore, recent studies suggest the presence of an unidirectional proton symport system in plastid membranes for the export of specific isoprenoid intermediates involved in the metabolic cross talk between cytosolic and plastidial pathways (Bick and Lange, 2003).

To investigate the biosynthesis of terpenes in carrots in more detail in vivo feeding experiments with [5,5-$^2H_2$]-mevalonic acid lactone (d_2-MVL) and [5,5-$^2H_2$]-1-deoxy-D-xylulose (d_2-DOX), the dephosphorylated precursor of the DOXP/MEP pathway, were used in this paper to study the intra-plant variation of cytosolic mevalonate and plastidial methylerythritol phosphate pathways. A separate investigation of phloem and xylem was carried out to localize the site of terpene biosynthesis and to clarify the question whether the presence of oil ducts is mandatory for terpene biosynthesis.

2. Results and discussion

For investigating the terpene biosynthesis in *D. carota* L. the cultivars Kazan and Bolero were used because of their relatively high terpene concentration. In carrot roots terpinolene and β-caryophyllene were chosen as representatives for mono- and sesquiterpenes, respectively. The volatiles, extracted by stir bar sorptive extraction (SBSE), were analyzed by thermal desorption–multidimensional gas chromatography–mass spectrometry (TD–MDGC–MS), allowing the simultaneous detection of genuine and labelled terpenes. They were identified by co-injection of commercial standards, retention times and mass spectra. As shown in Table 1, the concentrations of the genuine isoprenoids were clearly higher in phloem than in xylem in both cultivars. These results are in agreement with previous studies (Selanik and Simon, 1987; Habegger and Schnitzler, 1997).

However, terpinolene was not detectable in the xylem of cultivar Bolero. Labelled precursors were administered to the different root tissues simply by separating xylem and phloem manually. An aqueous solution of labelled MVL or DOX was directly incubated with phloem or xylem tissue. Following the incubation, the tissues were homogenized using a mortar and pestle and the metabolites were extracted as described above. The feeding experiments with labelled precursors and phloem tissue revealed an incorporation of d_2-DOX into terpinolene in both cultivars (see Table 1). Fig. 1 shows the SBSE-MDCG-MS analysis of terpinolene after administration of labelled DOX. Beside genuine unlabelled terpinolene labelled d_2- and d_4-terpinolene are clearly detectable and can be separated from unlabelled terpinolene due to the inverse isotope effect of deuterium labelled compounds in GC.

The degree of deuterium incorporation can be deduced from the M^+ peaks in the corresponding mass spectra (see Fig. 1). The M^+ peak of unlabelled terpinolene is shifted by two and four mass units in d_2- and d_4-terpinolene, respectively. The labeling pattern of d_2- and d_4-terpinolene can be deduced from the corresponding mass spectra. Unlabelled terpinolene shows intense mass peaks at 93 and 121 that can be assigned to the fragments shown in Fig. 1 (Thomas and Wilhalm, 1964). In labelled [3,3,5,5-$^2H_2$]-terpinolene these fragments should be

Table 1
Concentrations of genuine and labelled terpinolene and β-caryophyllene in μg/g plant root material as determined by SBSE

Terpene	Cultivar			
	Bolero		Kazan	
	Phloem	Xylem	Phloem	Xylem
d_2-DOX feeding				
d_0-Terpinolene	Trace	n.d.	15 ± 5	9 ± 1
$d_{2/4}$-Terpinolene	Trace	n.d.	51 ± 4	n.d.
d_0-Caryophyllene	168 ± 3	35 ± 7	90 ± 2	50 ± 4
$d_{2/4/6}$-Caryophyllene	82 ± 9	18 ± 7	42 ± 4	12 ± 4
d_2-MVL feeding				
d_0-Terpinolene	Trace	n.d.	26 ± 3	10 ± 4
$d_{2/4}$-Terpinolene	n.d.	n.d.	n.d.	n.d.
d_0-Caryophyllene	180 ± 4	Trace	89 ± 3	Trace
$d_{2/4/6}$-Caryophyllene	75 ± 4	17 ± 2	41 ± 1	29 ± 5

The results are given as average of three independent determinations ± standard deviation.
n.d., not detectable.

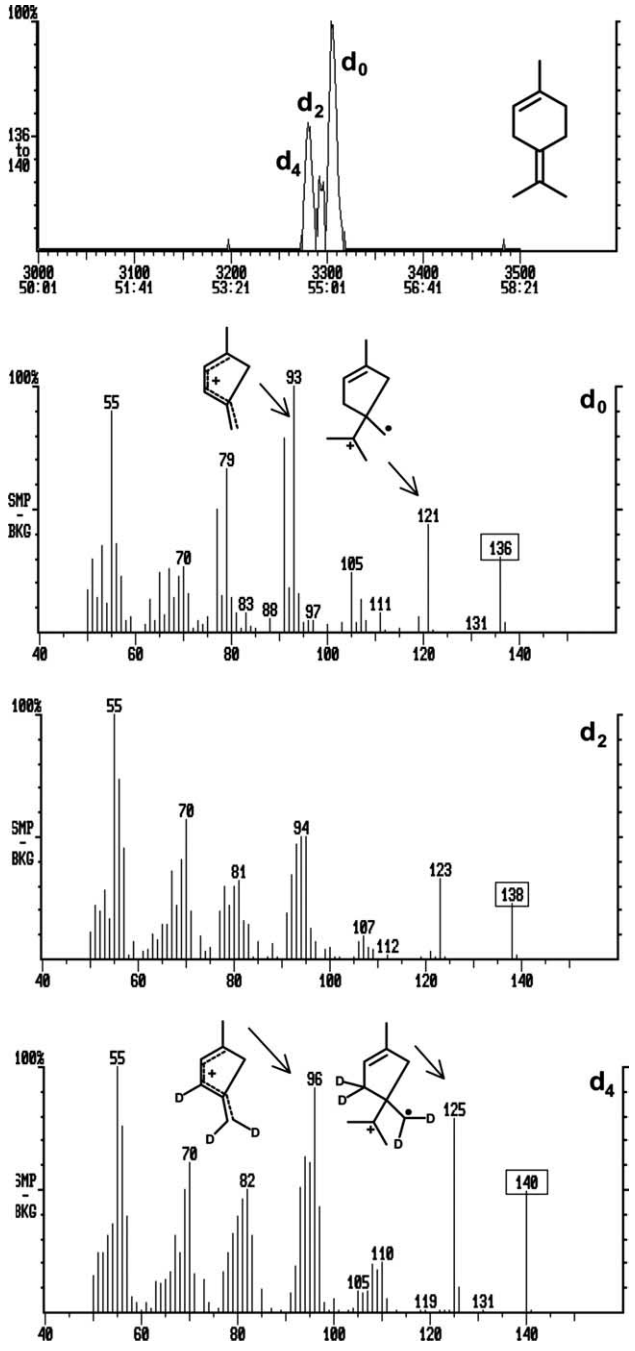

Fig. 1. Chiral main column chromatogram and MS spectra of unlabelled and labelled terpinolene obtained from a SBSE–MDGC–MS-analysis of *Daucus carota* L. cv. Kazan when [5,5-$^2H_2$]-DOX is administered to root phloem.

shifted by three and four mass-units, respectively, which indeed is the case. Thus, the labeling pattern found for d_4-terpinolene is in agreement with the incorporation of IPP and DMAPP units that were generated from DOX via the novel DOXP-pathway (see Fig. 3). The same applies for d_2-terpinolene. However, no incorporation into terpinolene was detectable in the xylem of both cultivars (see Table 1). In contrast to terpinolene, an incorporation of d_2-DOX into β-caryophyllene was detectable in xylem and phloem in both cultivars. In general, phloem tissue showed approximately four fold higher incorporation rates than xylem tissue, which explains the higher terpene concentration in the phloem of carrots. Feeding experiments with d_2-MVL showed that this precursor was exclusively incorporated into the sesquiterpene (see Fig. 2) but not into the monoterpene in the xylem and phloem of both cultivars.

The incorporation rates were comparable with those of labelled DOX and were higher in the phloem. Thus, the bulk of terpenes are certainly synthesized in the phloem tissue with a clearly smaller biosynthetic activity in the xylem tissue. Nevertheless, the detectable de novo-biosynthesis of β-caryophyllene in xylem tissue demonstrates that even in the absence of oil ducts a terpene biosynthesis is possible. It is noteworthy, that this biosynthetic activity in the xylem ceased completely when the carrots were stored for 7 days at 4 °C in contrast to the biosynthetic activity in phloem tissue (data not shown).

The assignment of d_6-β-caryophyllene in Fig. 2 has to be regarded as tentative due to the following reason. The mass spectrum of unlabelled β-caryophyllene shows key fragments at $m/z = 119$, 133 and 189 that are only shifted by 4 mass-units in labelled β-caryophyllene. Since β-caryophyllene is a sesquiterpene and thus assembled by 3 isoprene units a shift by 6 mass units should be visible, at least for the higher mass fragments like the M^+ ion. Unfortunately, the M^+ ion of labelled β-caryophyllene was not detectable, when EI ionization was employed for MS detection. Most probably a methyl group containing 2 deuterium atoms is eliminated during the first fragmentation step. The labelling degree still needs to be clarified by using the milder CI technique. Nevertheless, the incorporation of labelled DOX and MVL into β-caryophyllene is clearly proven.

The feeding experiments with labelled precursors and phloem tissue revealed an incorporation of d_2-DOX into terpinolene as well as into β-caryophyllene, whereas d_2-MVL was exclusively incorporated into the sesquiterpene. Thus, IPP and DMAPP utilized for biosynthesis of sesquiterpenes in carrot roots can be offered via the cytosolic MVA route as well as via the plastidial DOXP/MEP pathway. According to these results one can assume a transport of IPP and/or DMAPP from the plastids into the cytosol, where the biosynthesis of sesquiterpenes is usually localized. An export of geranyl diphosphate is conceivable as well. This exchange of precursors in carrot roots appears to be unidirectional and exclusively reserved to the biosynthesis of sesquiterpenes due to the lack of occurrence of labelled terpinolene during the d_2-MVL feeding experiments (see Table 1). Indeed, Bick and Lange (2003) could recently demonstrate that plastid membranes possess a unidirectional proton symport system for the export of specific

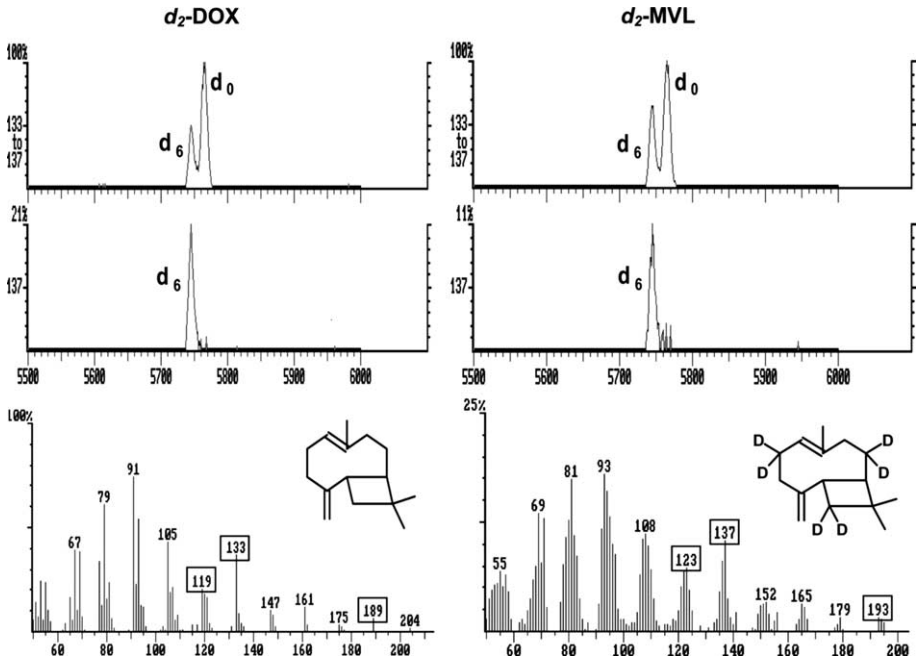

Fig. 2. Chiral main column chromatogram and MS spectra of unlabelled and labelled β-caryophyllene obtained from a SBSE–MDGC–MS-analysis of *Daucus carota* L. cv. Kazan when [5,5-$^2H_2$]-DOX and when [5,5-$^2H_2$]-MVL is administered to root phloem. The MS spectra shown are derived from the [5,5-$^2H_2$]-MVL feeding experiment. The corresponding MS spectra derived from the [5,5-$^2H_2$]-DOX feeding experiment are identical to those of the [5,5-$^2H_2$]-MVL feeding experiment.

isoprenoid intermediates involved in this metabolic cross talk. However, recently published findings for tobacco cells show that under rather restrictive conditions a complementation of plastidial isoprenoid synthesis by the cytosolic MVA pathway seems possible (Hemmerlin et al., 2003). A model for terpene biosynthesis in carrot roots on the basis of the results of the feeding experiments is shown in Fig. 3.

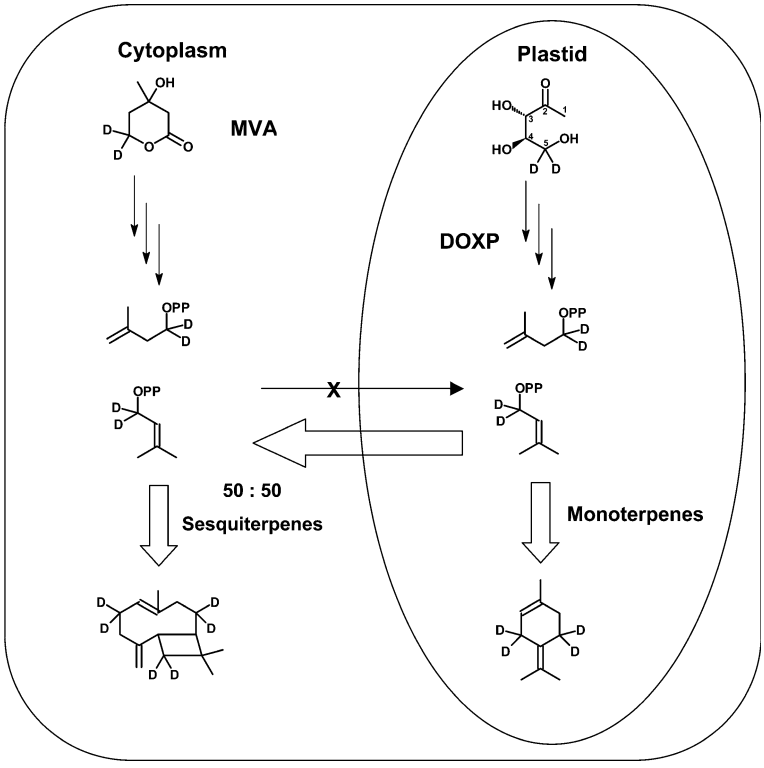

Fig. 3. Model of terpene biosynthesis in *Daucus carota* L.

Feeding experiments of the same design were also carried out with carrot leaves and myrcene and β-caryophyllene as representatives for monoterpenes and sesquiterpenes, respectively. Myrcene was chosen because it is the principal monoterpene in carrot leaves. Feeding experiments were only evaluated qualitatively without the determination of incorporation rates. The results are comparable to those that were obtained with roots. Labelled MVA is only incorporated into β-caryophyllene, whereas labelled DOX is incorporated into myrcene and β-caryophyllene. Labelled DOX and MVA were incorporated into β-caryophyllene at nearly equal rates as can be estimated from the peak areas of labelled β-caryophyllene (data not shown).

The in vivo feeding experiments revealed a biosynthetic activity in carrot leaves as well as in roots. Thus, both parts of the plant are able to generate terpenes independently from each other, which is in good agreement with the differences in terpene composition of carrot leaves and roots (Habegger and Schnitzler, 2000). The presence of certain terpenoids in either carrot leaves or roots confirm the findings of independent terpene biosynthesis in both tissues.

3. Conclusions

The in vivo feeding experiments with labelled precursors show a biosynthetic activity for mono- and sesquiterpenes in leaves as well as in roots of *D. carota* L. As far as the root is concerned, the biosynthesis of terpenes is mainly localized in the phloem. It seems reasonable to assume that these terpenes are mainly biosynthesized by the cells, which surround the oil ducts. A de novo biosynthesis of β-caryophyllene in the xylem, albeit at lower rates, could be demonstrated and shows that the presence of oil ducts is not a prerequisite for terpene biosynthesis in carrot roots. Both mono- and sesquiterpenes are biosynthesized via the novel DOXP/MEP route, whereas the MVA pathway is exclusively reserved for the biosynthesis of sesquiterpenes. This allocation of DOXP/MEP and MVA derived IPP/DMAPP resources for sequiterpene biosynthesis can be found in the xylem, phloem, and leaf tissue. It is remarkable that the origin of IPP/DMAPP used for sesquiterpene or sterol biosynthesis seems to be rather variable in the plant kingdom: Examples range from exclusive utilization of plastid-derived IPP/DMAPP for germacrene D biosynthesis (Steliopoulos et al., 2002) to almost exclusive utilization of MVA-derived IPP/DMAPP with only minor spillover of plastidial IPP units into cytosolic sterols (Arigoni et al., 1997). The results of this study for β-caryophyllene biosynthesis lie between these two extreme cases. Whether this allocation of resources can be influenced by stress factors or selective inhibitors is currently under investigation and will be published elsewhere.

4. Experimental

4.1. Plant material

Roots of *D. carota* L. cultivar Bolero and cultivar Kazan were obtained from the Research Centre Geisenheim (Geisenheim, Germany) in October 2003.

4.2. Chemicals

Decane and pentadecane, used as internal standards, were obtained from Fluka (Taufkirchen, Germany). Myrcene was obtained from Aldrich (Steinheim, Germany), terpinolene was obtained from Roth (Karlsruhe, Germany), and β-caryophyllene was obtained from Berje (Bloomfield NJ, USA). [5,5-$^2H_2$]-Mevalonic acid lactone was prepared according to Simpson et al. (1997), [5,5-$^2H_2$]-1-deoxy-D-xylulose was prepared according to Jux and Boland (1999). Spectral data of the labelled compounds were in all cases in good agreement with the data given in the references cited above.

4.3. Sample preparation

Carrot leaves were cut in small pieces and approximately 0.2 g were incubated with 1 ml of an aqueous feeding solution (d_2-MVL, 2 mg/ml; d_2-DOX, 2 mg/ml) for 4 days at room temperature in darkness.

Xylem was manually separated from phloem and both parts were cut in equal pieces (approximately $10 \times 2 \times 2$ mm). 1 g of plant material was incubated with 1ml of an aqueous feeding solution (d_2-MVL, 2 mg/ml or d_2-DOX, 2 mg/ml) for 4 days at room temperature in darkness. After removing the feeding solution the carrot pieces were ground up in 2 ml of phosphate buffer (pH 7) to a suspension, which was used for extraction.

Blank values were done by grinding up pieces in pure buffer. Decane and pentadecane were added as internal standards. Each experiment was repeated at least three times.

4.4. SBSE-sampling

A stir bar consisting of a magnetic core sealed inside a glass tube with a length of 1.2 cm, 1.2 mm o.d. and coated with 55 μl polydimethylsiloxane (PDMS) was used. Stir bars are manufactured and offered by Gerstel (Mühlheim/Ruhr, Germany). The SBSE conditions were as follows: stirring time 30 min at room temperature at approximately 450 rpm for homogeneous distribution of the components on the PDMS-phase.

4.5. Instrumental methods

^{1}H NMR spectra of the synthesized products were recorded on a Bruker AMX 300, 300 MHz, at room

temperature in $CDCl_3$/TMS or D_2O. The chemical shifts are given in δ (ppm).

The GC–MS analyses of the synthesized products were carried out on a Fisons Instruments GC 8065, coupled to a Fisons Instrument MD 800 mass spectrometer, equipped with a self-prepared fused silica capillary column coated with SE-52 (30 m × 0.25 mm i.d.; film thickness 0.23 μm). GC conditions: carrier gas helium 69 kPa; split 20 ml/min; injector temperature 230 °C; oven temperature 40 °C (5 min isothermal), then 5 °C/min to 260 °C (20 min isothermal); ion source temperature 200 °C; mass range 40–300 amu; EI 70 eV. The molecular ions (M^+) and fragment ions are given as *m*/*z* with relative peak intensities in % of the most abundant peaks.

The TD–MDGC–MS (Kreck et al., 2001) consists of a Gerstel TDS thermal desorption system, mounted on a Siemens SiChromat 2–8, with two independent column oven programs and a live T-switching device, coupled to the transfer line of a Finnigan MAT ITD 800 mass spectrometer, using an open split interface. For the thermal desorption the following conditions were applied: desorption temperature program, 10 °C at 60 °C/min to 250 °C, 2 min isothermal (6.0 min); flow mode TDS, splitless; transfer line temperature set at 250 °C. A Gerstel CIS-3 PTV injector was used for cryogenic focusing of the released analytes.

The PTV was cooled to −150 °C using liquid nitrogen. The PTV was programmed from −150 °C at 12 °C/s to 250 °C, 2 min isothermal (2.5 min). Flow mode CAS was splitless (1 min). The liner was filled with Tenax TA (Alltech, Deerfield, IL).

GC conditions were as follows: precolumn, self-prepared fused silica capillary coated with SE-52 (30 m × 0.25 mm i.d.; film thickness 0.23 μm); carrier gas helium, 1.9 bar; detector, FID, 250 °C. Main column: self-prepared fused silica capillary (30 m × 0.25 mm i.d.) coated with a 0.23 μm film of 4‰ heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (DIME-β-CD) (30%) in SE-52 (70%); detector, ITD 800; transfer line temperature 250 °C; open split interface, 250 °C; helium sweeping flow, 1 ml/min; ion trap manifold, 200 °C; EI, 70 eV; oven temperature program, precolumn, 60 °C (5 min isothermal), raised at 3 °C/min to 250 °C (20 min isothermal); main column, 60 °C (30 min isothermal), then 1 °C/min to 200 °C (15 min isothermal); cut times: decane/myrcene, 16.2–18.6 min; terpinolene, 21.0–23.4 min; β-caryophyllene 37.9–40.3; pentadecane, 40.9–43.3 min.

Acknowledgements

The authors gratefully thank Dr. Mirjam Kreck, Department of Wine and Beverage Research, Geisenheim, for making carrots available.

References

Alasalvar, C., Grigor, J.M., Quantick, P.C., 1998. Method for the static headspace analysis of carrot volatiles. Food Chem. 65, 391–397.

Arigoni, D., Sagner, S., Latzel, C., Eisenreich, W., Bacher, A., Zenk, M.H., 1997. Terpenoid biosynthesis from 1-deoxy-D-xylulose in higher plants by intramolecular skeletal rearrangement. Proc. Nat. Acad. Sci. USA 94, 10600–10605.

Bick, J.A., Lange, B.M., 2003. Metabolic cross talk between cytosolic and plastidial pathways of isoprenoid biosynthesis: unidirectional transport of intermediates across the chloroplast envelope membrane. Arch. Biochem. Biophys. 415, 146–154.

Croteau, R., Burbott, A.J., Loomis, W.D., 1973. Enzymatic cyclization of neryl pyrophosphate to α-terpineol by cell-free extracts from pepeprmint. Biochem. Biophys. Res. Commun. 50, 1006–1012.

Eisenreich, W., Rohdich, F., Bacher, A., 2001. Deoxyxylulose phosphate pathway to terpenoids. Trends Plant Sci. 6, 78–84.

Eisenreich, W., Bacher, A., Arigoni, D., Rohdich, F., 2004. Biosynthesis of isoprenoids via the non-mevalonate pathway. Cell. Mol. Life Sci. 61, 1401–1426.

Habegger, R., Schnitzler, W.H., 1997. Die Verteilung von aromagebenden Inhaltsstoffen in der Möhre. Die Industrielle Obst- und Gemüseverwertung 82, 39–42.

Habegger, R., Schnitzler, W.H., 2000. Aroma compounds in the essential oil of carrots (*Daucus carota* L. ssp. *sativus*). 1. Leaves in comparison with roots. J. Appl. Bot. 74, 220–223.

Hemmerlin, A., Hoeffler, J.F., Meyer, O., Tritsch, D., Kagan, I.A., Grosdemange-Billiard, C., Rohmer, M., Bach, T.J., 2003. Cross-talk between the cytosolic mevalonate and the plastidial methylerythritol phosphate pathways in tobacco bright yellow-2 cells. J. Biol. Chem. 278, 26666–26676.

Jux, A., Boland, W., 1999. Improved protocol towards isotopically labelled 1-deoxy-D-xylulose. Tetrahedron Lett. 40, 6913–6914.

Kjeldsen, F., Christensen, L.P., Edelenbos, M., 2001. Quantitative analysis of aroma compounds in carrot (*Daucus carota* L.) cultivars by capillary gas chromatography using large-volume injection technique. J. Agric. Food Chem. 49, 4342–4348.

Kjeldsen, F., Christensen, L.P., Edelenbos, M., 2003. Changes in volatile compounds of carrots (*Daucus carota* L.) during refrigerated and frozen storage. J. Agric. Food Chem. 51, 5400–5407.

Kreck, M., Scharrer, A., Bilke, S., Mosandl, A., 2001. Stir bar sorptive extraction (SBSE)–enantio-MDGC–MS – a rapid method for the enantioselective analysis of chiral flavour compounds in strawberries. Eur. Food Res. Technol. 213, 389–394.

Lichtenthaler, H.K., Schwender, J., Disch, A., Rohmer, M., 1997a. Biosynthesis of isoprenoids in higher plant chloroplasts proceeds via a mevalonate-independent pathway. FEBS Lett. 400, 271–274.

Lichtenthaler, H.K., Rohmer, M., Schwender, J., 1997b. Two independent biochemical pathways for isopentenyl diphosphate and isoprenoid biosynthesis in higher plants. Physiol. Plant. 101, 643–652.

Lichtenthaler, H.K., 1999. The 1-deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants. Annu. Rev. Plant Mol. Biol. 50, 47–65.

Luan, F., Wüst, M., 2002. Differential incorporation of 1-deoxy-D-xylulose into (3*S*)-linalool and geraniol in grape berry exocarp and mesocarp. Phytochemistry 60, 451–459 (and references cited therein).

Rohmer, M., 1999. The discovery of a mevalonate-independent pathway for isoprenoid biosynthesis in bacteria, algae and higher plants. Nat. Prod. Rep. 16, 565–574.

Selanik, D., Simon, P.W., 1986. Relationship between oil ducts and volatile terpenoid content in carrot roots. Am. J. Bot. 73, 60–63.

Selanik, D., Simon, P.W., 1987. Quantifying intra-plant variation of volatile terpenoids in carrot. Phytochemistry 26, 1975–1979.

Shine, W.E., Loomis, W.D., 1974. Isomerization of geraniol and geranyl phosphate by enzymes from carrot and peppermint. Phytochemistry 13, 2095–2101.

Simpson, T.J., Ahmed, S.A., McIntyre, R., Scott, F.E., Sadler, I.H., 1997. Biosynthesis of polyketide-terpenoid (meroterpenoid) metabolites andibenin B and andilesin A in *Aspergillus variecolor*. Tetrahedron 53, 4013–4034.

Steliopoulos, P., Wüst, M., Adam, K.P., Mosandl, A., 2002. Biosynthesis of the sesquiterpene germacrene D in *Solidago canadensis*: ^{13}C and ^{2}H labeling studies. Phytochemistry 60, 13–20.

Thomas, A.F., Wilhalm, B., 1964. Les spectres de masse des hydrocarbures monoterpeniques. Helv. Chim. Acta 47, 475–488.