Differential incorporation of 1-deoxy-D-xylulose into monoterpenes and carotenoids in higher plants

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1-Deoxy-D-xylulose labelled with ^{13}C or ^{14}C is incorporated into the monoterpenoids menthone, menthol, menthofuran, eucalyptol, pulegone, geraniol and thymol by higher plants, however, the rate of incorporation was lower as compared with incorporation into carotene and phytol.

Recent evidence indicates that 1-deoxy-D-xylulose (DX) or a derivative such as the 5-phosphate is the committed precursor for the biosynthesis of isoprenoids in some bacteria.[1,2] The deoxyxylulose pathway is also operative in higher plants.[3–9] Thus, pigments of life, such as chlorophyll and carotenoids are formed in plants from the pentose derivative and not from mevalonate.[7,8] Recent incorporation experiments with ^{13}C-labelled glucose have also shown that essential oils in plants are formed *via* the alternative pathway.[9]

In order to study in more detail the involvement of DX in monoterpenoid biosynthesis, we prepared [1-^{13}C]- and [2-^{13}C]DX by condensation of D-glyceraldehyde with [3-^{13}C]- and [2-^{13}C]pyruvate, respectively, using pyruvate dehydrogenase as catalyst.[10,11] The spectral characteristics of the deoxypentulose obtained were identical to published values.[12] [1,2-$^{14}C_2$]DX was obtained similarly from [U-^{14}C]pyruvate (yield 75–83%; spec. act., 62.5 μCi μmol^{-1}).

Shoots of peppermint (*Mentha x piperita*) with a length of about 5 cm were cut and were allowed to form roots in hydroponic culture. A solution containing 27 μM [1,2-$^{14}C_2$]DX was applied to the rooted shoots for 48 h. The monoterpenes were obtained by steam distillation, and the metabolites were isolated as described earlier.[9] Relative specific activities of 0.1% for menthone, 0.2% for menthol, 0.3% for menthofuran and 0.3% for eucalyptol were observed. In similar experiments, *Mentha pulegium* showed incorporation into pulegon (0.2%), *Pelargonium graveolens* into geraniol (0.3%), and *Thymus vulgaris* into thymol (0.2%).

^{13}C-Labelled DX samples were supplied to *M. piperita* shoots at a concentration of 25 mM. Menthone was isolated[9] and was subjected to NMR analysis as described earlier.[7] Relative ^{13}C abundance of individual carbon atoms was calculated from the NMR signal integrals of biosynthetic samples by comparison with the natural abundance sample (% ^{13}C in Table 1). The values were referenced to 1.10% for the carbon with the lowest ^{13}C enrichment. In the experiment with [1-^{13}C]DX, the carbon atoms 7 and 9 of menthone were enriched with 1.49 and 1.41% ^{13}C abundance, respectively. The other atoms were virtually unlabelled with 1.16 ± 0.06% ^{13}C abundance. On the other hand, [2-^{13}C]DX labelled the carbon atoms 1 and 8 of menthone with 1.39 and 1.41% ^{13}C abundance, whereas the other atoms had a ^{13}C abundance of 1.20 ± 0.05% (Table 1). On the basis of the established isoprene dissection of the cyclic monoterpene it follows that [1-^{13}C]DX enriched C-5 of IPP/DMAPP and that [2-^{13}C]DX enriched C-3 of IPP/DMAPP, respectively (Fig. 1). This labelling pattern is in full accordance with terpenoid formation *via* the deoxyxylulose pathway.

It should be noted that the observed incorporation rates of DX into the monoterpenes under study are low. However, they exceed the rates achieved with labelled acetate or mevalonate by factors of at least 10 to 100 fold.[13] This indicates that the deoxyxylulose pathway is the dominant metabolic route for essential oil formation. Based on the incorporation of ^{13}C- and ^{14}C-labelled DX and on our previous [^{13}C]glucose feeding experiments using the same plants,[9] we conclude that the deoxyxylulose pathway leading to isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) is operative in essential oil formation, which is at variance with previous reports.[14]

In experiments using *M. piperita* it was observed that [1,2-$^{14}C_2$]DX was incorporated with considerably higher rates into chlorophyll a and b (13%) and into β-carotine (10%) than into the monoterpenes. This is in accordance with incorporation rates of DX into chlorophylls and carotenoids in a cell culture of *Catharanthus roseus*.[7] The different incorporation rates of DX into monoterpenes and into chlorophylls/carotenoids could indicate that an enzyme involved in the utilization of exogenous DX (*e.g.* a kinase) is limiting in the plant glandular trichomes which have been recognized as sites of monoterpenoid biosynthesis,[14,15] but not in the chloroplasts where chlorophylls and carotenoids are synthesized. Limited transport of labelled DX into the plant glandular trichomes might also explain the discrepancy of incorporation rates.

Table 1 ^{13}C NMR analysis of menthone from *Mentha x piperita* supplied with ^{13}C labelled 1-deoxyxylulose (DX). Values indicative for IPP formation *via* the deoxyxylulose pathway are shown in bold type. The signal with lowest ^{13}C enrichment (marked by asterisk) was referenced to 1.1% ^{13}C abundance

		Proffered precursor	
Position	δ^b	[1-^{13}C]DX % ^{13}C	[2-^{13}C]DX % ^{13}C
1	35.3	1.10*	**1.39**
2	50.5	1.18	1.21
3	212.3	1.10	1.10*
4	55.5	1.12	1.15
5	27.4	1.18	1.19
6	33.5	1.16	1.20
7	21.9	**1.49**	1.25
8	25.4	1.12	**1.41**
9	18.3	**1.41**	1.23
10	20.9	1.29	1.27

[a] For signal assignments ref. 16. [b] Referenced to $CDCl_3$ solvent signal at 77.0 ppm.

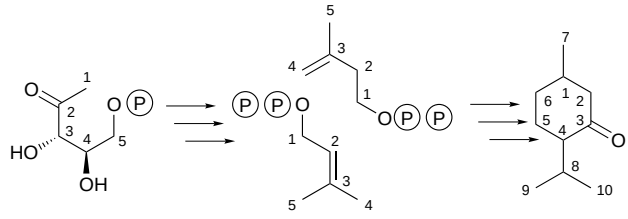

Fig. 1

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Footnote and References

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