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Biosynthesis of 2-C-Methyl-D-erythritol in Plants by Rearrangement of the Terpenoid Precursor, 1-Deoxy-D-xylulose 5-Phosphate

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Abstract:

Leaves of *Liriodendron tulipifera* convert 1-deoxy-D-xylulose to 2-C-methyl-D-erythritol via a skeletal rearrangement reminiscent of the formation of terpene precursors from 1-deoxy-D-xylulose. The data suggest that 2-C-methyl-D-erythritol 4-phosphate is either an intermediate or a side product of the deoxyxylulose 4-phosphate pathway of terpenoid biosynthesis. © 1998 Elsevier Science Ltd. All rights reserved.

In animals, the universal terpenoid precursors, dimethylallyl pyrophosphate (**7**, DMAPP) and isopentenyl pyrophosphate (**6**, IPP), are biosynthesized via the mevalonate pathway which has been studied in considerable detail (for review see Ref. 1). More recently, it has been shown that certain eubacteria synthesize the isoprenoid precursors by a different route via 1-deoxy-D-xylulose 5-phosphate (**3**) which is generated from pyruvate (**1**) and glyceraldehyde 3-phosphate (**2**) by the catalytic action of 1-deoxyxylulose 5-phosphate synthase (Fig. 1) [2]. Higher plants predominantly utilize the mevalonate pathway for the biosynthesis of sterols and the deoxyxylulose pathway for the biosynthesis of hemiterpenes, monoterpenes, diterpenes, phytol and carotenoids [3].

Experiments using multiply ^{13}C labelled 1-deoxy-D-xylulose indicated that the carbon skeleton of the linear pentulose is modified by a skeletal rearrangement during conversion to the branched chain isoprenoid precursors **6** and **7** (Fig. 1) [3]. Specifically, the C-3/C-4 bond of 1-deoxy-D-xylulose is broken, and a new bond is formed between C-2 and C-4 of the original deoxypentulose intermediate. The observed intramolecular rearrangement in the metabolism of **3** has precedent in pinacol-type rearrangements involved in the biosynthesis of the riboflavin precursor, 3,4-dihydroxy-2-butanone 4-phosphate [4], of the valine precursor, acetolactate [5], and of the branched-chain carbohydrate, apiose [6]. By analogy with these reactions, the direct product resulting from the rearrangement of **3**, which has yet to be isolated, could be 2-C-methyl-

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D-erythrose 4-phosphate (**4**, Fig. 1). However, the details of the deoxyxylulose pathway are still unknown.

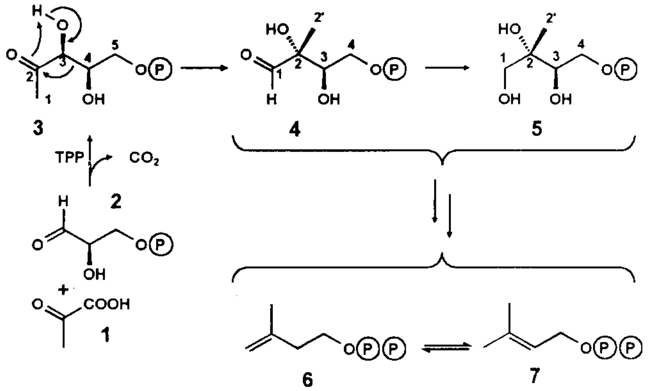

Fig. 1. Biosynthesis of the terpenoid precursors isopentenyl pyrophosphate (6) and dimethylallyl pyrophosphate (7) via the deoxyxylulose pathway. TPP, thiamine diphosphate.

The reduction of the hypothetical branched carbohydrate **4** could yield 2-C-methyl-D-erythritol 4-phosphate (**5**). The dephosphorylation product of **5**, 2-C-methyl-D-erythritol (**9**), has been found in substantial amounts in phylogenetically different plant species [7-11]. A corresponding oxidation product, 2-C-methyl-1,4-erythronolactone, has been isolated from the iberian milk-vetch *Astragalus lusitanicus* [12]. A 2,4-cyclopyrophosphate of the branched polyol has been observed as a stress metabolite in *Corynebacterium ammoniagenes* and other bacteria [13]. The biosynthesis of 2-C-methylerythritol-2,4-cyclopyrophosphate has been studied in the bacterium, *C. ammoniagenes*, by Rohmer and his coworkers using various ^{13}C labelled isotopomers of glucose [14]. The observed labelling patterns in the carbon skeleton of the polyol were equivalent to those of the isoprenoid units of dihydromenaquinone. Moreover, the incorporation of ^{2}H-labelled 2-C-methylerythritol into the isoprenoid side-chains of ubiquinone and menaquinone from *E. coli* was observed by Rohmer and coworkers [15] and was verified by us (unpublished).

In order to analyze the biosynthetic origin of 2-C-methylerythritol (**9**) in plants, we performed labelling experiments with *Liriodendron tulipifera* and *Ipomoea parasitica* using 1-deoxy-D-xylulose (**8**) labelled with ^{13}C or ^{14}C. [1-^{13}C]- and [2,3,4,5-$^{13}C_4$]-**8** were synthesized as described earlier [3] and were applied to detached leaves of *L. tulipifera*. Specifically, 10 leaves (approximately 5 g dry weight) were allowed to take up 66 mg of [1-^{13}C]-**8** or 50 mg of [2,3,4,5-$^{13}C_4$]-**8** for a period of 49 h at 22°. The leaves were extracted with boiling water. The extracts were concentrated under reduced pressure. The residue was subjected to paper chromatography using n-butanol/pyridine/H_2O/acetic acid (60:40:30:3, v/v) as solvent (R_f of 2-C-methylerythritol, 0.62). The 2-C-methylerythritol fraction was recovered and was subjected to thin layer chromatography on silica plates using CH_2Cl_2/ethanol (7:3, v/v) as solvent (R_f of 2-C-methylerythritol, 0.55). One- and two-dimensional ^{13}C NMR experiments were performed as described earlier [16].

^{13}C NMR signals of **9** from the labelling experiment with [1-^{13}C]-**8** are shown in Fig. 2A. The ^{13}C enrichment of the C-2' methyl carbon is immediately obvious from the relative intensities of the signals and is also reflected in the ^{13}C coupled satellites of C-2. The ^{13}C abundance of

C-2' was calculated as 16.9 %, whereas the other carbon atoms were unlabelled (Table). It follows that label from [1-^{13}C]-**8** was diverted regiospecifically to C-2' of **9** with surprisingly high enrichment rates (Fig. 3).

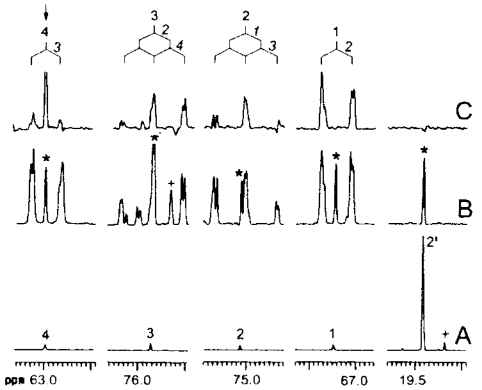

Fig. 2. ^{13}C NMR signals of 2-C-methylerythritol from *L. tulipifera*. A, after incorporation of [1-^{13}C]1-deoxyxylulose; B, after incorporation of [2,3,4,5-$^{13}C_4$]1-deoxyxylulose; signals reflecting natural ^{13}C abundance isotopomers are indicated by asterisks; signals arising from impurities are indicated by +; C, ^{13}C-TOCSY experiment after incorporation of [2,3,4,5-$^{13}C_4$]1-deoxyxylulose; selective excitation of C-4 (270° gaussian pulse of 10 ms length) followed by a 32-ms TOCSY mixing period (MLEV-17, preceded and followed by trim pulses of 3 ms length, spin lock field of 5 kHz strength). ^{13}C coupling patterns as analysed by INADEQUATE experiments (Table) are indicated; atoms coupled to the respective index carbon are indicated in italics.

The ^{13}C NMR signals of **9** from the labelling experiment with [2,3,4,5-$^{13}C_4$]-**8** were characterized by a singlet for C-2' and by complex multiplets caused by ^{13}C^{13}C coupling for C-1, C-2, C-3, and C-4 (Fig. 2B). The quantitative analysis of signal integrals revealed that C-1, C-2, C-3 and C-4 of **9** had a ^{13}C-abundance of 8.2 ± 0.2 % (Table). An one-dimensional ^{13}C TOCSY experiment with selective excitation of C-4 showed magnetization transfer extending to C-1 at the other end of the polyol chain (Fig. 2C). Similarly, selective excitation of C-4 followed by TOCSY mixing resulted in magnetization transfer to C-3, C-2 and C-1. This documented the presence of an isotopomer with an uninterrupted sequence of 4 contiguous ^{13}C atoms (i.e. C-1, C-2, C-3, C-4). The formation of formal [1,2,3,4-$^{13}C_4$]-**9** from [2,3,4,5-$^{13}C_4$]-**8** proves the strictly intramolecular nature of a rearrangement process reminiscent of the mechanism observed in IPP and DMAPP formation in a cell culture of *Catharanthus roseus* (Fig. 3) [3].

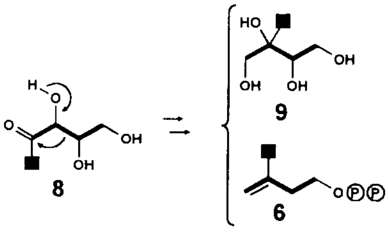

Fig. 3. Skeletal rearrangement conducive to the observed labelling patterns of 2-C-methylerythritol (**9**) from *L. tulipifera* after incorporation of ^{13}C labelled 1-deoxy-xylulose (**8**) and of IPP (**6**) as reconstructed from phytol, β-carotene and luteine in *C. roseus* [3]. ^{13}C labels from [1-^{13}C]-**8** are shown as solid squares. Contiguous labelling from [2,3,4,5-$^{13}C_4$]-**8** is shown by bold lines.

In parallel to the experiments described above, leaves of *L. tulipifera* and *I. parasitica* were fed with [1,2-^{14}C]-**8** [3]. The incorporation rates into **9** and β-carotene were 19 % and 8 %, respectively, using leaves of *L. tulipifera*, and 19 % and 4 %, respectively, using leaves of *I. parasitica*. The data show that 1-deoxy-D-xylulose is a common precursor of 2-C-methylerythritol and β-carotene in *L. tulipifera* and *I. parasitica*.

We propose that exogenous 1-deoxy-D-xylulose is converted to the 4-phosphate (**3**) by a plant kinase and subsequently to **5** via **4** by isomerization and reduction. **5** could then be converted to IPP and DMAPP and used for terpenoid syntheses. In certain plants, **5** is dephosphorylated and 2-C-methylerythritol is accumulated. Rohmer and his coworkers proposed **5** as a committed precursor of IPP and DMAPP [14, 15]. On the other hand, the incorporation of ^{2}H labelled 2-C-methylerythritol into the isoprenoid side chain of menaquinone was low in *E. coli* [15], and ^{14}C- or ^{2}H-labelled 2-C-methylerythritol could not be incorporated into terpenoids of the plant *C. roseus* (unpublished). The reason for these apparent inconsistencies may be due to insufficient incorporation rates of the branched chain polyol or due to insufficient action of a necessary kinase. On the other hand, it is possible that **4** is the committed precursor of IPP and that 2-C-methylerythritol is a side product of the terpenoid pathway.

Table. ^{13}C NMR analysis of 2-C-methylerythritol obtained from leaves of *L. tulipifera* supplied with ^{13}C labelled 1-deoxyxylulose (**8**).

Position	δ^{a}	$J_{CC}{}^{b}$	Supplement			
	ppm	Hz	[1-^{13}C]-**8**	[2,3,4,5-$^{14}C_4$]-**8**		
			% $^{13}C^{c}$	% $^{13}C^{c}$	% $^{13}C^{13}C^{d}$	INADEQUATE
1	67.24	39.8(2)	1.1	8.2	83.8	2
2	75.06	39.4(2')	1.2	8.2	nd[e]	1,3
2'	19.42	39.4(2)	16.9	1.1	8.2	
3	75.86		1.1	8.3	nd[e]	2,4
4	62.97	39.2(3)	1.1	8.1	84.2	3

[a] ^{13}C chemical shift referenced to external 3-trimethylsilylpropane 1-sulfonate; signal assignments according to Ref. 17.
[b] $^{13}C^{13}C$ coupling constants; the coupled atom to the respective index atom is indicated in parentheses
[c] ^{13}C abundance , calculated from ^{13}C NMR signal integrals
[d] fraction of $^{13}C^{13}C$-coupled satellites in the global ^{13}C signal of the respective index atom
[e] Not determined due to signal overlapping

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