Stereochemical course of the reduction step in the formation of 2-*C*-methylerythritol from the terpene precursor 1-deoxyxylulose in higher plants

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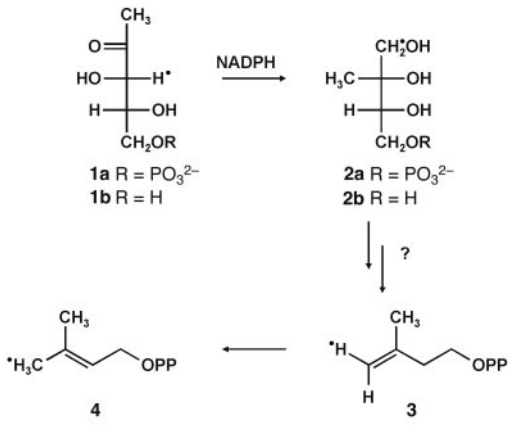
Feeding of 1-deoxy-D-[3-^{2}H]xylulose to leaves of the tree *Liriodendron tulipifera* affords 2-*C*-methyl-*D*-erythritol labelled specifically in the H_{Si} position of C-1.

Over the last few years evidence has been accumulated in different laboratories for the existence in eubacteria, algae and higher plants of an alternative mevalonate-independent metabolic pathway for the formation of isopentenyl pyrophosphate (IPP, **3**) and dimethylallyl pyrophosphate (DMAPP, **4**), the two universal building blocks of terpene biosynthesis (Scheme 1).[1] The first C_5 intermediate in this pathway, 1-deoxyxylulose 5-phosphate **1a**, is assembled from pyruvic acid and glyceraldehyde 3-phosphate in a decarboxylative reaction that requires the participation of thiamine pyrophosphate as a cofactor, and genes specifying for the corresponding synthase have been cloned from *Escherichia coli*[2] and from *Mentha piperita*.[3] The isolation of a NADPH-dependent reductoisomerase from *E. coli* capable of catalyzing the transformation of **1a** into 2-*C*-methylerythritol 4-phosphate **2a** has been described recently.[4] While the remaining steps of the sequence remain unknown, it has been shown that the (*E*)-methyl group of DMAPP **4** and the terminal methylene group of IPP **3** acquire label from 1-deoxy[3-^{2}H]xylulose **1b** (H• = D) in *E. coli*[5] as well as in cell cultures of *Catharanthus roseus*.[6] In addition, the specific localization of this label in the H_E position of the IPP generated in the plant system rules out DMAPP as the committed precursor of IPP along the deoxyxylulose pathway.[6] Supporting evidence for the same conclusion has been obtained in work with secretory cells from *M. piperita*.[7]

The well documented efficiency of leaves of the tree *Liriodendron tulipifera* in converting exogenous 1-deoxyxylulose **1b** into 2-*C*-methylerythritol **2b**[8] was exploited in the present study for investigating the stereochemical course of the reduction step of this transformation in a feeding experiment involving the easily accessible 1-deoxy[3-^{2}H]xylulose **1b** (H• = D).[9] For this purpose the leaf stems were immersed into a 18.5 mM solution of the precursor at room temperature and under natural daylight illumination. After two days the desired product was isolated from the biomass as described.[8] Following careful purification by HPLC on a column of Rezex Phenomenex (300 × 7.8 mm), using distilled water as eluent and a refractometer as the detecting system, the biosynthetic sample was converted into the known bis-acetonide **5**.[10]† Assignments of all the ^{1}H and ^{13}C NMR signals were obtained for an unlabeled reference specimen of **5** by two dimensional homonuclear (COSY, NOESY) and heteronuclear (HMQC) experiments. The data are summarized in Table 1 and Fig. 1. Specifically, the assignment of the ^{1}H NMR signals at δ 3.64



Scheme 1

Table 1 NMR data of 1,2:3,4-di-*O*-isopropylidene-2-*C*-methylerythritol **5** in $CDCl_3$. The transmitter frequency for ^{1}H was 500.13 MHz

Position	δ_H (mult, *J*/Hz)	δ_C
1 (*Re*)	3.64 (d, 8.7)	72.89
1 (*Si*)	3.95 (d, 8.7)	
2	—	80.96
2′	1.26 (s)	19.18
3	4.00 (t, 6.5)	78.37
4 (*Re*)	3.80 (dd, 8.7, 5.9)	65.35
4 (*Si*)	3.94 (dd, 8.6, 7.2)	
5-CH_3 (*Re*)	1.27 (s)	26.49
5-CH_3 (*Si*)	1.28 (s)	27.15
6-CH_3 (*Re*)	1.32 (s)	26.09
6-CH_3 (*Si*)	1.23 (s)	24.61
5	—	109.47[a]
6	—	109.51[a]

[a] Assignments may be interchanged.

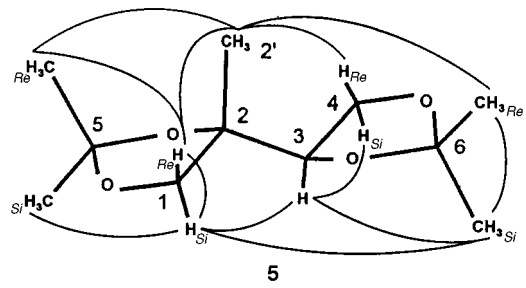


Fig. 1 Predominant conformation of **5** as reconstructed from two-dimensional NOESY experiments. Observed interactions are indicated by curved lines.

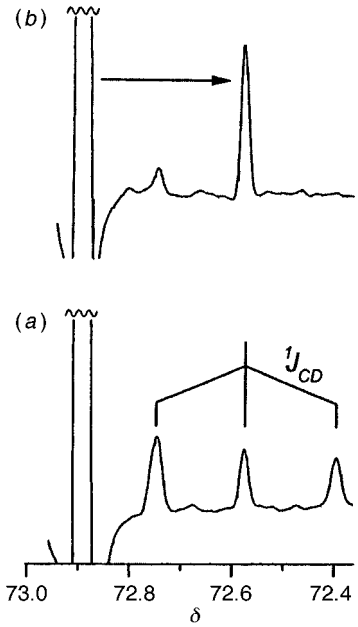


Fig. 2 ^{13}C NMR signals of C-1 in **5** derived from the feeding experiment: (*a*) ^{1}H-decoupled, (*b*) ^{1}H,^{2}H-decoupled.

and 3.95 to H_{Re} and H_{Si} of C-1, respectively, is well-supported by the network of NOE interactions detailed in Fig. 1. Monodeuteration at C-1 in the sample of **5** derived from the feeding experiment was evidenced by the appearance in the proton-decoupled ^{13}C NMR spectrum of a new triplet (J_{CD} = 22.5 Hz) with an upfield shift of −313 ppb from the normal signal at δ 72.89 (Fig. 2a). Under ^{2}H-decoupling conditions this triplet collapsed to a singlet (Fig. 2b) with an intensity of 4% relative to the overall intensity of the C-1 signal. In addition, the ^{2}H NMR spectrum of the compound displayed a single signal at δ 3.96, thus ensuring that the deuterium was specifically localized in the H_{Si} position of C-1.

On the basis of this evidence the stereochemical course of the conversion **1b** → **2b** can now be depicted as in Fig. 3a (R = H). The reaction is likely to occur through the intermediacy of the corresponding phosphoric acid monoesters (R = PO_3^{2-}).[4] Comparison with the mechanistically related steps involved in the biosynthesis of valine and isoleucine[11] (*cf.* Fig. 3b) reveals a close stereochemical matching, which suggests that in each of the reactions there is a steric necessity for the migrating group and the reducing cofactor to be located on opposite faces of the planes defined by the respective hydrogen-bridged α-hydroxycarbonyl substructures.

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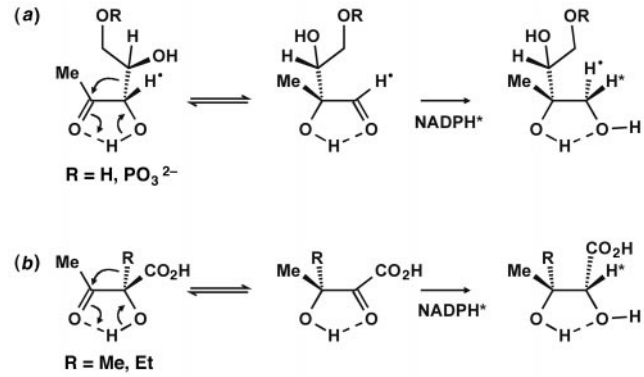

Fig. 3 (*a*) Stereochemical course of the 1-deoxyxylulose conversion into 2-*C*-methylerythritol. (*b*) stereochemical course of reductoisomerase reactions in the biosynthesis of valine (R = Me) and isoleucine (R = Et).

Notes and references

† In improvement of the original procedure a solution of 21 mg of **2b** in 8 ml of acetone was treated with 2 ml of 2.2 M $ZnCl_2 \cdot Et_2O$ in CH_2Cl_2 at room temperature for 24 h; after addition of 20 ml of $CHCl_3$ the organic phase was washed with $NaHCO_3$ (5%) and water, dried with $MgSO_2$ and concentrated to a crude oil, which was purified on a column of silica gel (0.5 × 10 cm) with hexane–EtOAc (3 : 1 v/v) as the solvent to yield pure **5** in a yield of 77%.

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