Is Isoprene Emitted by Plants Synthesized via the Novel Isopentenyl Pyrophosphate Pathway?

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The incorporation of deuterium labeled 1-deoxy-D-xylulose into isoprene, emitted by three higher plants upon illumination, was analysed by headspace GC-MS. A high extent of incorporation into isoprene was found, indicating that isoprene is synthesized in higher plants via the novel isopentenyl pyrophosphate pathway, of which 1-deoxy-D-xylulose-5-phosphate is an intermediate. A new chemical synthesis of methyl [1-$^2H_1$]-1-deoxy-α/β-D-xyluloside from D-xylulose is presented.

Introduction

Isoprene (C_5H_8) is emitted by many plants in amounts of about 175 to 452 × 10^{12} g C per year world-wide (Rasmussen and Khalil, 1988; Zimmermann, 1979; Dignon and Logan, 1990; Taylor, 1990; Müller, 1992; for reviews see: Sharkey, 1996; Sharkey and Singsaas, 1995; Sharkey *et al.*, 1991). It can scavenge hydroxyl radicals very quickly and is therefore an essential component in atmospheric chemistry, especially for tropospheric ozone formation (Trainer, 1987; Chameides *et al.*, 1988; Fehsenfeld *et al.*, 1992). Isoprene is generated light-dependent (Sharkey and Loreto, 1993) by an isoprene synthase from dimethylallyl pyrophosphate (DMAPP) (Wildermuth and Fall, 1996; Schnitzler *et al.*, 1996; Silver and Fall, 1995; Kuzma and Fall, 1993), which is in equilibrium with isopentenyl pyrophosphate (IPP, Fig. 1). There is some indication that the biosynthesis of isoprene in plants may proceed within the chloroplast compartment inasmuch as there exists a thylakoid-bound isoprene synthase (Wildermuth and Fall (1996) and refs. cited therein). As of now, however, there is no proof for this possible origin of isoprene from the chloroplast compartment.

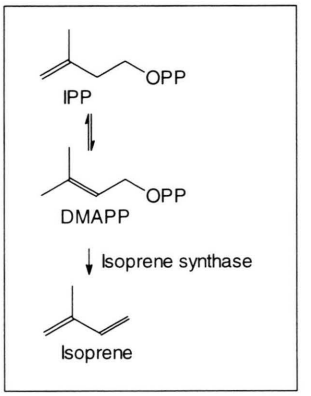

Fig. 1. Biosynthesis of isoprene from IPP and DMAPP via the isoprene synthase.

Isoprene biosynthesis is generally thought to follow the classical acetate/mevalonate pathway via IPP, the common precursor for all isoprenoids (for reviews see: Spurgeon and Porter (1981); Goodwin T. W. (1977). Label from [^{14}C]-mevalonate was, however, only poorly incorporated into the isoprene emitted (Jones and Rasmussen,

Abbreviations: DMAPP, dimethylallyl pyrophosphate; IPP, isopentenyl pyrophosphate; GAP, glyceraldehyde 3-phosphate; PGA, 3-phosphoglyceric acid; GC-MS, combined gas chromatography/mass spectrometry; RubisCO, ribulose-1,5-bisphosphate carboxylase/oxygenase; P_N, net CO_2 assimilation; Rfd values, variable chlorophyll fluorescence decrease ratio.

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1975). Recently it was shown that the plastidic isoprenoids β-carotene, lutein, the phytol side chain of chlorophyll and the solanesyl side chain of plastoquinone-9 in *Hordeum vulgare* L., *Daucus carota* L. and *Lemna gibba* L. are synthesized via IPP formation through a novel mevalonate-independent biosynthetic pathway (Lichtenthaler *et al.*, 1996). This new pathway, first uncovered in some eubacteria (Rohmer *et al.*, 1993) and later also shown to occur in the green alga *Scenedesmus obliquus* (Schwender *et al.*, 1997), starts with the aldol-type addition of glyceraldehyde 3-phosphate (GAP) onto a C_2-unit of pyruvate to produce 1-deoxy-D-xylulose-5-phosphate. The latter is then converted in several steps to IPP under rearrangement of the carbon skeleton (Fig. 2). In *E. coli*, which also exhibits the alternative IPP pathway, deuterium labeled 1-deoxyxylulose was shown to be incorporated into the isoprenoid side chain of ubiquinone (Broers, 1994). The generation of diterpenes of *Gingko* and *Taxus* was also shown to proceed via a non-mevalonate route (Schwarz, 1994; Eisenreich *et al.*, 1996), similarly pointing towards the novel GAP/pyruvate pathway. The various enzymic steps and intermediates between 1-deoxy-D-xylulose-5-phosphate and IPP as well as the exact cofactor requirements are not clearly known, yet for theoretical considerations one ATP and several NADPH are likely to be required.

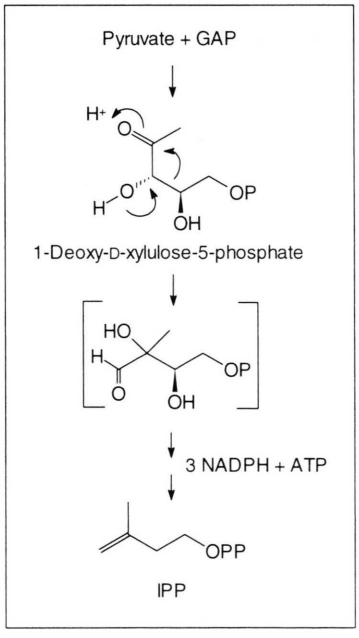

Fig. 2. *De novo* biosynthesis of IPP according to the non-mevalonate GAP/pyruvate pathway recently discovered (Rohmer *et al.*, 1993; Schwender *et al.*, 1996), conceivably proceeding via 1-deoxy-D-xylulose-5-phosphate and a C-methyl-branched D-tetrose-4-phosphate as key intermediates.

Since all plastidic isoprenoids studied hitherto are formed via this new IPP biosynthetic pathway, it can be assumed that isoprene too, if synthesized within the chloroplast, is produced via this GAP/pyruvate IPP pathway. In order to obtain more information on the biosynthesis of the isoprene emitted by plant leaves, we decided to study the possible incorporation of labeled 1-deoxy-xylulose, a key intermediate in this new IPP pathway, into isoprene. Labeled methyl 1-deoxy-D-xyluloside was surmised to be a more favorable substrate than the free pentulose, as it would be much more readily taken up by the plant and then hydrolyzed to the free sugar by glycosidases in the plant tissue. As a result we report here the synthesis of deuterium-labeled methyl [1-$^2H_1$]-1-deoxy-α/β-xyluloside and its incorporation into the isoprene emitted by leaves of three higher plants upon illumination.

Materials and Methods

Methyl [1-$^2H_1$]-1-deoxy-α/β-D-xyluloside (**1**)

The deuterium-labeled 1-deoxy-D-xyluloside (1-deoxy-D-*threo*-pentuloside) was synthesized from D-xylulose via its known 2,3-*O*-isopropylidene-β-D-xylulose **2**, readily accessible from D-xylose by treatment with hot pyridine (→ D-xylulose) and subsequent acetonation (Hough and Theobald, 1962). Exposure of **2** to p-toluenesulfonyl chloride in pyridine at -15 °C resulted in regioselective tosylation of the more reactive primary hydroxyl group to give **3**, in which the tosyloxy moiety was displaced by reaction with $LiAlD_4$ (→ **4**). Acid-promoted methanolysis of **4** smoothley effected removal of the isopropylidene group and formation of the methyl 1-deoxy-xyluloside **1**, isolated as a colorless syrup upon chromatographic purification (Fig. 3).

2,3-O-Isopropylidene-1-O-tosyl-β-D-xylulofuranose (**3**)

A solution of 10.0 g (8.67 mmol) p-toluenesulfonyl chloride in 35 ml of chloroform was added

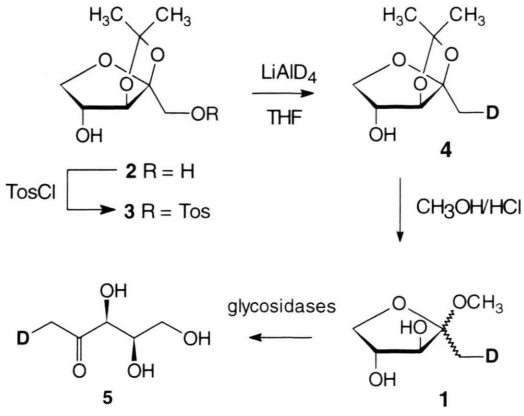

Fig. 3. Chemical synthesis of the applied deuterium labeled methyl 1-deoxy-α/β-D-xyluloside (**1**) from compound **2** via **3** and **4.** (THF = tetrahydrofuran). Plant glycosidases readily hydrolize **1** to the free sugar which in solution preferentially exists in the open-chain tautomeric form **5.**

dropwise, with stirring to a cooled (-15 °C) solution of 1.65 g (8.67 mmol) of 2,3-*O*-isopropylidene-β-D-xylulofuranose (**2**, m.p. 70–71 °C, Hough and Theobald, 1962) in 20 ml of pyridine containing 4 Å molecular sieve (2 g). After stirring for 15 h at -15 °C the solution was diluted with 20 ml of $CHCl_3$, filtered through kieselgur and flash-evaporated under reduced pressure. Crystallization from 2-propanol yielded 1.73 g (58%) of **3** as colourless crystals; m.p. 122 °C; $[\alpha]_D^{20} = +14.3°$ (c=1, $CHCl_3$). – ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.32, 1.46 (2s, 6H, 2 CH_3), 2.36 (d, 1H, 4-OH), 2.45 (d, 1H CH_3), 4.13–4.28 (m, 5H, 1-H_2 , 4-H, 5-H_2), 4.45 (s, 1H, 3-H), 7.34, 7.80 (2d, 4H, C_6H_4), $J_{4\text{-}OH,4\text{-}H}$ = 6.32 Hz. – ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 21.8, 26.3, 27.8, (3 CH_3), 68.6 (C-5), 74.4 (C-1), 75.2 (C-4); 85.4 (C-3), 111.9 (**C**$(CH_3)_2$), 128.1–130.1 (C_6H_4). – MS (FD, 10mA): m/z: 344 $[M]^+$, 345 $[M+1]^+$, 329 $[M\text{-}CH_3]^+$. – C_{15} H_{20} O_7 S (344.38): calcd. C 52.32 , H 5.85 , S 9.31 ; found C 52.27 , H 5.79 , S 9.19.

[1-$^2H_1$]-1-Deoxy-2,3-O-isopropylidene-β-D-xylulofuranose (**4**)

A solution of 700 mg (2.0 mmol) of **3** was stirred under reflux with 150 mg (6 mmol) of $LiAlD_4$ in 20 ml of THF for 12h. The mixture was cooled to -5 °C, quenched with methanol (10 ml), filtered through kieselgur and cautiously flash-evaporated under reduced pressure. Column chromatography on alumina with dichloromethane-acetone (20:1, 2.5 × 24 cm) afforded **4** as a colourless syrup. Sublimation at 100 °C under vacuum yielded 238 mg (67%) of **4** as colourless crystals; m.p. 62 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.31 and 1.43 (two 3H-s, 2 CH_3), 1.64 (s, 2H, CH_2D), 2.53 (d, 1H, 4-OH), 4.07 and 4.10 (two , 1H-dd, 5-H_2), 4.17 (s, 1H, 3-H), 4.21 (d, 1H, 4-H), $J_{4,5}$ = 2.2, $J_{5,5}$ = 10.2 Hz, – ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 24.6 (3 CH_2D), 29.3, 31.7 (3 CH_3), 73.4 (C-5), 75.9 (C-4), 87.6 (C-3), 111.1 (C-2), 113.6 (**C**$(CH_3)_2$). – MS (FI) m/z: 176 $[M+1]^+$, 160 $[M\text{-}CH_3]^+$. – C_8 H_{14} DO_4 (176.2): calcd. C 54.53, H 8.01; found C 54.47, H 7.99.

Methyl [1-$^2H_1$]-1-deoxy-D-xylulofuranoside (**1**)

To a methanolic solution of **4** (1.1 g, 6.2 mmol in 25 ml) was added dropwise 2 M HCl (0.8 ml), followed by stirring of the mixture at 50 °C for 8 h. Subsequent neutralization with a basic anion exchanger, filtration through kieselgur and flash evaporation under reduced pressure gave a residue, which was purified by elution from a silica gel column (1.5 × 20 cm, deactivated with 3% water) with dichloromethane-methanol and chloroform-methanol (90:10 and 80:20) to afford 740 mg (87%) of **1** as a colorless syrup, comprising an approximate 3:2 mixture of α- and β-anomers. – ^{1}H NMR (390 MHz, D_4-methanol): δ 1.33 and 1.37 (two 1H-m, 1-H_2), 3.24 and 3.34 (2s in 3:2 ratio, total intensity 3H, α- and β-OCH_3), 3.53 and 4.10 (two 1H-m, 5-H_2), 3.66 (d, 0.4 H, $J_{3,4}$ = 5.9 Hz, 3H of **1β**), 3.79 (d, 0.6 H, $J_{3,4}$ = 2.0 Hz, 3H of **1α**), 3.98 (dd, 0.6H, 4-H of **1α**), 4.24 (ddd, 0.4 H, 4-H of **1β**). – MS (FD, 10mA): m/z = 149 $[M]^+$.

Exposure of either **1** or **4** to 2M HCl in acetonitril (24 h, 25 °C) – conditions used previously for the deblocking of 3,4-*O*-isopropylidene-1-deoxy-D-xylulose (Kennedy et al., 1995) – gave [1-$^2H_1$]-1-deoxy-D-xylulose as an approximate 4:1:1 tautomeric mixture of open chain form **5** (2H-s for 1-H_2 at 2.21 ppm) and the furanoid anomers (1-H_2 signals at 1.36 and 1.40, in D_2O, resp.).

Plant material

Green photosynthetically active leaves of celandine (*Chelidonium majus* L.) and willow (*Salix viminalis* L.) were obtained from plants grown in the Botanical Garden of the University of Karls-

ruhe. Leaves of poplar (*Populus nigra* L.) were taken from a tree on the local University campus and from rooted cuttings grown in the greenhouse.

Gas exchange measurements were performed with a CO_2/H_2O-porometer (Walz, Effeltrich, Germany).

Chlorophylls and carotenoids were analysed according to Lichtenthaler (1987).

Administration of the precursor methyl [1-$^2H_1$]-1-deoxy-D-xyluloside (**1**) *and mass spectrometric analysis of isoprene*

Verification and isotopic analysis of isoprene emission was carried out by a method modified after Monson and Fall (1989): Leaves of the three species mentioned above were cut under water at their petiole and placed instantly into a solution of 3 to 6 mg methyl[1-$^2H_1$]-1-deoxy-D-xyluloside in 150 to 400 µl tap water in a 4 ml glass vial. In another experiment 5 mg [2-^{13}C]-acetate (sodium salt) dissolved in 400 µl water was applied. To ensure a good uptake of the precursor the leaf's transpiration was promoted by passing a light stream of air over the vial up to several hours at 25 °C. From time to time small amounts of water were added to the vial in order to keep the petiole in water and replace water that had been taken up into the leaf or partly been evaporated. Thereafter the vial was sealed with a teflon coated silicone septum. After an incubation under light of high irradiance (1500 µmol m^{-2} s^{-1}) for 30 to 80 min at room temperature the septum was pierced with a GC syringe containing 2 µl decane. The decane was then pressed out of the syringe needle as a hanging drop and exposed to the vial airspace for 15 to 45 min to achieve an enrichment of the emitted gaseous isoprene in the decane droplet. After that the drop was pulled back into the syringe and quickly injected into a Hewlett-Packard 5890 Series II GC coupled with a Hewlett-Packard 5971A Mass Selective Detector. In some cases a probe of charcoal was placed into the vial and the adsorbed isoprene was eluted and injected into the GC. The gas chromatograph was equipped with a fused silica capillary (20m × 0.32 mm) coated with Carbowax 20M. Temperature programming was 70 °C for 2 min, then at 20 °C/min to 200 °C.

The procedure of illumination, decane extraction and GC-MS analysis was repeated a few times with the same leaf. After each time the vial was opened to remove the emitted isoprene by flowing an air stream through the vial. The most intense incorporation of deuterium from methyl[1-$^2H_1$]-1-deoxy-D-xyluloside was usually observed about 20 to 24 h after placing the leaf petiole into the solution of the deuterium labeled precursor. If isoprene emitted after longer time periods (at 30, 36 or 48 h) was analysed, deuterium label was still found, but the degree of labeling continuously declined towards the natural isotopic distribution. Authentic isoprene (Fluka) showed the same retention time and fragmentation pattern as the isoprene of plant origin. Without an illumination of high irradiance no isoprene emission could be detected by the GC-MS technique.

Results

The leaves of the three plants celandine, poplar and willow, investigated for their light-induced isoprene emission, were fully green and exhibited the usual chlorophyll and carotenoid composition of leaves with a functioning photosynthetic apparatus (Table I). The leaves were photosynthetically active as shown by regular and relatively high values of the variable chlorophyll fluorescence ratio (Table II) measured at 690 nm and 735 nm (Rfd690 and Rfd735 values), a ratio which functions as a vitality index of the photosynthetic apparatus (Lichtenthaler and Rinderle, 1988; Babani and Lichtenthaler, 1996). Also the photosynthetic net CO_2 assimilation rates P_N indicated full photosynthetic function of the leaves. The P_N-rates, however, declined somewhat at a longer (30 min) high irradiance exposure of 1500 µmol m^{-2} s^{-1} as

Table I. Content of total chlorophylls *a+b* and carotenoids *x+c* (in mg m^{-2} leaf area) and weight ratios of pigments Chl *a/b* and chlorophylls to carotenoids *(a+b)/(x+c)* in green leaves of celandine (*Chelidonium*), poplar (*Populus*) and willow (*Salix*). Mean of 6 values from 2 different plants (*Chelidonium*) and 6 different leaves from 2 branches each (*Salix, Populus*). Standard deviation of pigments were ± 6% and of pigment ratios ± 4%.

Pigments	*Chelidonium*	*Populus*	*Salix*
Chlorophylls, $a + b$	528	430	402
Carotenoids, $x + c$	105	102	95
Chl *a/b*	2.9	3.7	2.9
$(a + b)/(x + c)$	5.0	4.2	4.2

Table II. Values of the variable chlorophyll fluorescence decrease ratio, Rfd690 and Rfd735 (as vitality index of the photosynthetic apparatus), rates of net photosynthetic CO_2 fixation P_N (μmol CO_2 m^{-2} s^{-1}) and stomatal conductance for water vapour (gH_2O-values in mmol m^{-2} s^{-1}) in green attached leaves of different plants measured at high irradiance (1500 μmol m^{-2} s^{-1}). Mean of 8 values from 4 different leaves (Rfd-values, standard deviation ± 5%) and 2 leaves (net CO_2 assimilation, deviation ± 8%).

	Chelidonium	*Populus*	*Salix*
Rfd690	4.18	4.00	2.80
Rfd735	2.96	2.76	1.84
P_N after 15 min light	-*	3.16	3.73
P_N after 30 min light	-*	2.96	3.43
gH_2O in low light	-*	98	147
gH_2O after 15 min high light	-*	39	79
gH_2O after 30 min high light	-*	33	58

* not determined.

compared to the 15 min P_N-rates (Table II). This was induced by a partial closure of the stomata at the high irradiance of 1500 μmol m^{-2} s^{-1} as seen in the declining gH_2O values from 15 to 30 min high-light exposure (Table II).

After illumination of the celandine (*Chelidonium majus)* leaf in the closed vial for 30 min at 1500 μmol m^{-2} s^{-1}, considerable amounts of the emitted isoprene were absorbed from the vial air by the hanging drop of the organic solvent in the subsequent 15 min and could easily be detected by GC-MS (Fig. 4, unlabeled). When this leaf was kept in darkness or at low light conditions (20 to 100 μmol m^{-2} s^{-1}), no isoprene could be detected. The isoprene emission proceeded at the first high-light exposure, and the emission process could be repeated several times with the same leaf up to 48 h.

When labeled methyl deoxyxyluloside was applied to the leaf (via the petiole) several hours before the illumination, deuterium from [1-$^2H_1$]-1-deoxy-D-xylulose was incorporated to a high degree into the emitted isoprene as shown in the mass spectrum (Fig. 4, labeled) which was measured after an incorporation time of the precursor of 24 h. Due to the incorporation of the labeled precursor the relative abundance of the mass peak 67 had decreased, whereas that of 68 and 69 were significantly increased and a small peak of mass 70 showed up which was not present in the control.

The mass spectrum of unlabeled isoprene exhibits its two major fragments, 68 $[M]^+$ and 67 $[M-H]^+$.

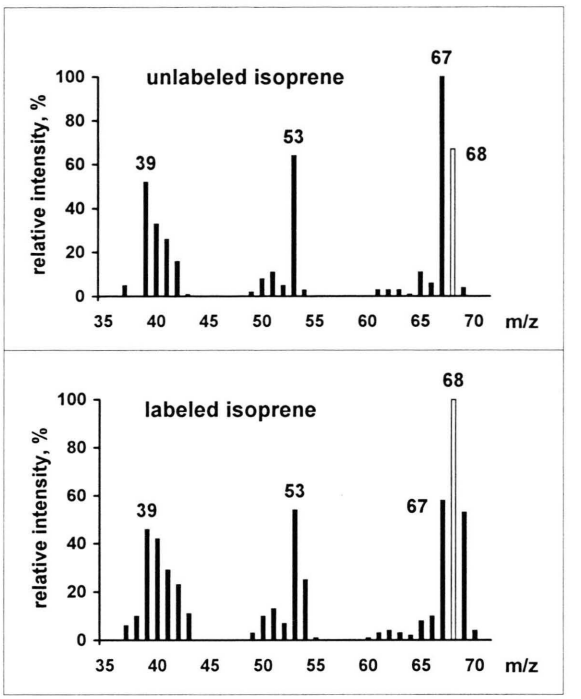

Fig. 4. Mass spectra of unlabeled isoprene and of deuterium labeled isoprene emitted from leaves of *Chelidonium majus* 24 h after administration of a methyl[1-$^2H_1$]-1-deoxy-D-xyluloside solution via the petiole. The isoprene emitted was labeled to ca. 60% from the applied [1-$^2H_1$]-1-deoxy-D-xylulose.

Isoprene molecules which are labeled with ^2H will yield the same fragmentation, but the fragments will be one mass unit heavier (69 $[M]^+$, 68 $[M-H]^+$, the less abundant fragment 67 $[M-^2H]^+$ is not considered here). On this basis we predicted the possible mass spectra of isoprene under assumption of different degrees of incorporation of [1-$^2H_1$]-1-deoxy-D-xylulose. The calculated mass spectra were compared with the actually measured spectra, and in this way the percentage of deuterium label in isoprene could be estimated. Figure 5 shows the peak cluster in the region of the molecular ion of isoprene, calculated under the premise, that 10 to 70% of the isoprene molecules are derived from [1-$^2H_1$]-1-deoxy-D-xylulose. By comparison of the mass peaks of the celandine experiment (Fig. 4, labeled) with the calculated distributions of mass peaks shown in Fig. 5, one can estimate that ca. 60% of the emitted isoprene

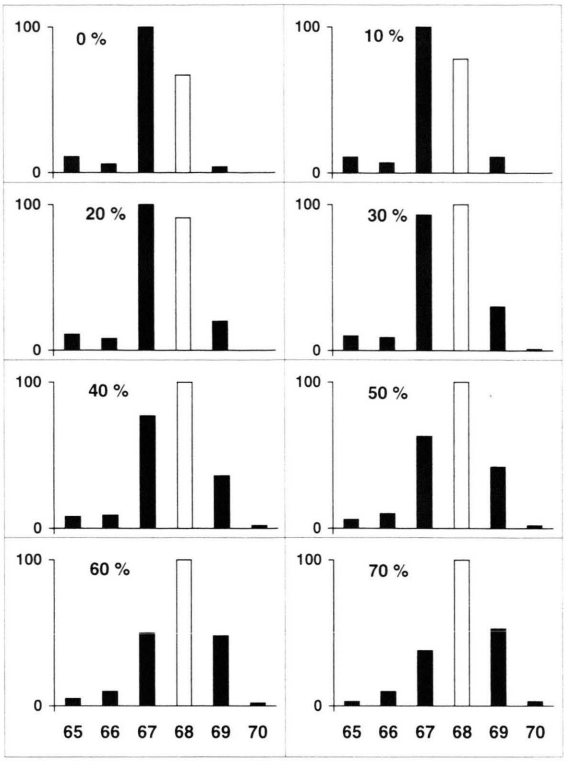

Fig. 5. Calculation of the change in the distribution of mass peaks of isoprene (range 65 to 70) for an assumed incorporation of 10 to70% of deuterium from [1-$^2H_1$]-1-deoxy-D-xylulose.

was labeled from the applied deuterium-deoxyxylulose. Such high labeling degrees were only reached after an incubation and uptake time of the precursor substance to the leaf of more than 16 h.

In further experiments with green photosynthetically active poplar (*P. nigra*) and willow (*S. viminalis*) leaves, the high-light induced isoprene emission could also be demonstrated in these species using our detection approach. In both cases we could demonstrate the incorporation of deuterium label from [1-$^2H_1$]-1-deoxy-D-xylulose into the emitted isoprene. In both species the extent of incorporation of labeled deoxyxylulose into the emitted isoprene was estimated in the range of 40 to 50% (cf. Fig. 5).

In order to obtain further information on the time-dependent uptake of deuterium-labeled methyl deoxyxyluloside into the leaf and its incorporation into the emitted isoprene, we studied in poplar leaves the labeling of emitted isoprene in the course of 30 h after application of the labeled precursor. After 4 h of incorporation a significant labeling of the isoprene could be detected. The labeling degree rose with a maximum near 20 h (Fig. 6). Thereafter the labeling degree of the emitted isoprene declined and was significantly lower at 25 h and 30 h after methyl[1-$^2H_1$]-1-deoxy-D-xyluloside application via the leaf petiole.

In one experiment with poplar leaves we supplied [2-^{13}C] acetate in solution via the petiole and checked the possible incorporation of ^{13}C-label into the isoprene emitted at high-light conditions. In contrast to the control leaf, where after 24 h 40 to 45% of the emitted isoprene were labeled by the applied [1-$^2H_1$]-1-deoxy-D-xylulose, we found extremely low rates of ^{13}C-incorporation from [2-^{13}C] acetate into isoprene (16 h after application less than 2%, after 24 h less than 8% and after 36 h less than 10%). Considering the fact that via the acetate/mevalonate pathway the ^{13}C-atoms from three acetates should have been incorporated into one isoprene, the ^{13}C-incorporation degree into isoprene from [2-^{13}C]-acetate were much lower than is indicated by the above percentage values.

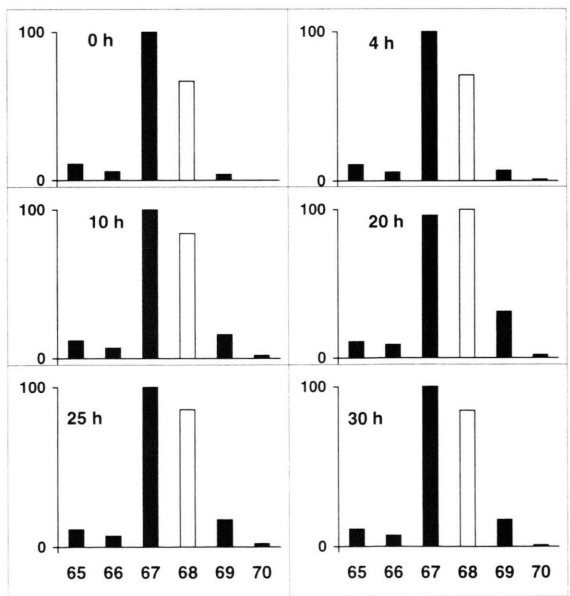

Fig. 6. Time dependent labeling of isoprene emitted by a poplar leaf from [1-$^2H_1$]-1-deoxy-D-xylulose at different times (4 to 30h) after application of the precursor to the leaf.

Discussion

1-Deoxy-D-xylulose-5-phosphate is the proposed intermediate of the newly detected alternative IPP biosynthesis pathway from which all chloroplast isoprenoids tested so far (carotenoids, phytol of chlorophylls, C45-prenyl side chain of plastoquinone-9) are generated (Schwender *et al.*, 1996; Lichtenthaler *et al.*, 1997). The incorporation of 1-deoxyxylulose into plastidic isoprenoids such as the phytol side chain was also shown for duckweed (*Lemna gibba* L.), whereas the cytosolic sterols, which are formed via the acetate/mevalonate pathway, were poorly labeled (Schwender *et al.*, unpublished results 1996). The high degree of incorporation of the applied methyl [1-$^2H_1$]-1-deoxy-D-xyluloside into the isoprene, emitted under high-light conditions in the 3 plants tested here with maximum values of 40 to 60%, indicates that 1-deoxyxylulose is also a good and direct precursor of the isoprene emitted by leaves under photosynthesis conditions. A shortage of CO_2 in the closed vial system during illumination of the leaves may have favoured the high degree of incorporation of the offered intermediate into the emitted isoprene. The molecular mass of unlabeled isoprene is 68. Most, if not all, labeled isoprene molecules carried only one deuterium atom evidenced by the high abundance of mass 69 versus the low abundance of mass 70. This is indication, that [1-$^2H_1$]-1-deoxy-D-xylulose is incorporated into isoprene as a whole molecule. If some of it had been broken down and metabolized indirectly (e. g. via acetyl-CoA and mevalonic acid, according to the hitherto assumed biosynthesis pathway for isoprene formation) one would have expected another distribution of the isoprene ions.

Isoprene is emitted under high light conditions, whereby up to 20% of the photosynthetically fixed CO_2 is reemitted as isoprene (e. g. Sharkey and Loreto, 1993). Sharkey *et al.* (1991) discussed the possibility of isoprene biosynthesis preventing overreduction of the photosynthetic apparatus and electron carriers under high-light conditions. They calculated the energetic cost of isoprene formation via pyruvate, acetyl-CoA and mevalonate, on the basis of the hitherto assumed isoprene biosynthesis pathway, assuming the involvement of 9 CO_2, 14 NADPH and 27 ATP, as well as the release of 4 CO_2. They also suggested that isoprene might be built up from pyruvate generated by RubisCO since it was shown that RubisCO can yield during photosynthesis in 1% of the carboxylation events one pyruvate plus one PGA molecule instead of two PGA molecules (Andrews and Kane, 1991). Sharkey *et al.* (1991) concluded, however, that isoprene formation might not be a suitable sink for dissipating excess ATP and NADPH, because the ratio NADPH/ATP was lower than for CO_2 assimilation (0.67). In view of the new alternative pathway for isoprene formation, however, this ratio would be about 0.8 and thus be considerably higher. In any case and independent of any value of the NADPH/ATP ratio, when the newly discovered IPP forming pathway operates for isoprene formation in the chloroplast, as it does for carotenoids, the phytol of chlorophylls, and the prenyl chain of plastoquinone (Lichtenthaler *et al.*, 1997), the formation of isoprene would serve as a sink for NADPH and ATP, and thus help to protect the photosynthetic apparatus with its reaction centers at high irradiances against photoinhibition and photooxidative damage. Since isoprene can serve as a scavenger of highly reactive oxygen species such as hydroxyl radicals (Fehsenfeld *et al.*, 1992), isoprene formation in chloroplasts would have a double protective function for the photosynthetic apparatus.

Earlier labeling experiments had shown that ^{14}C-labeled acetate and mevalonate were incorporated into isoprene at very low rates only (Jones and Rasmussen, 1975), leaving in doubt the presumable precursor function of both substrates. Further evidence for the non-mevalonate origin of isoprene is provided by our observation that in poplar leaves ^{13}C-acetate was very poorly incorporated into the emitted isoprene as well as by early investigations of other plastidic isoprenoids, e. g. in maize which showed β-carotene to be efficiently labeled from [^{14}C]-pyruvate but not from [^{14}C]-acetate (Shah and Rogers, 1969). All these results can be explained by the operation of the mevalonate-independent IPP pathway in plastids of higher plants which uses GAP, pyruvate and the resulting 1-deoxy-D-xylulose-5-phosphate as precursors (Fig. 2). The high extent of labeling of phytogenous isoprene by a precursor of this new IPP pathway supports the view of the isoprene being synthesized in the chloroplasts, and this is in agreement with the recent finding of a plastidic isoprene synthase (Wil-

dermuth and Fall, 1996). The ready labeling of emitted isoprene from applied and photosynthetically fixed $^{13}CO_2$ (Delwiche and Sharkey, 1993) also favours a plastidic formation of isoprene. Because phytogenous isoprene is a good model system for IPP biosynthesis, investigations are being performed with other precursors with which we hope to obtain further insight into the biosynthetic sequence of the GAP / pyruvate IPP pathway and isoprene formation in higher plants.

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