8: 38%; m.p. 306–307 °C; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 7.90$ (br. s, 4H, NH), 7.73 (d, 4H, fluorene-H), 7.38 (m, 12H, fluorene-H), 5.89 (t, 4H, pyrrole-H_β), 5.53 (t, 4H, pyrrole-H_β), 1.49 (s, 12H, CH_3); ^{13}C NMR (50.03 MHz, $CDCl_3$): $\delta = 148.4$, 140.1, 139.7, 132.6, 127.8, 127.4, 124.7, 120.4, 106.3, 103.7, 55.6, 35.9, 29.8; MS (CI): m/z: 673.0 $[M+H]^+$; C,H,N analysis: calcd for $C_{48}H_{40}N_4 \cdot H_2O$: C 83.45, H 6.13, N 8.11; found: C 83.56, H 6.15, N 8.0.

Crystal structure determination: Crystallographic data were collected by using a Philips PW 1100 four-circle, computer-controlled, diffractometer by using graphite crystal monochromated $Mo_{K\alpha}$ radiation ($\lambda = 0.7107$ Å) with $\omega/2\theta$ scan (scan width: 1.2°, scan rate: 3° min^{-1}). Unit cell parameters were determined by a least squares fitting of the setting angles of 25 centered reflections. The intensities of three standard reflections were measured every 120 min during the data collection. No absorption correction was performed. All non-hydrogen atoms were found by direct methods (SHELXS-86) and refined anisotropically (SHELXL-93). Hydrogen atoms were found by using a difference Fourier map and refined isotropically. The structures were solved by direct methods and refined on $|F_o|^2$. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC-101785 (**6**) and CCDC-101786 (**4**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

Crystal data for **4:** 293 K, clear crystals, crystal dimensions $0.45 \times 0.35 \times 0.30$ mm, trigonal, $R\bar{3}$, $a = 23.75(3)$, $b = 23.75(3)$, $c = 24.20(3)$ Å, $V = 11822(26)$ Å^3, $Z = 18$, $\rho_{calcd} = 1.083$ g cm^{-1}, $\mu = 0.13$ mm^{-1}, $I > 2\sigma(I)$, $F(000) = 4092$, 2θ range 4.3–24.08, 4329 reflections collected, 2718 independent, 289 refined parameters, $\Delta\rho_{max} = 0.205$, $wR = 0.1807$, $R_{obs} = 0.092$.

Crystal data for **6:** 298 K, clear crystals, crystal dimensions $0.30 \times 0.22 \times 0.15$ mm, monoclinic, $P2_1/n$ (No. 14), $a = 12.080(4)$, $b = 14.320(4)$, $c = 14.690(4)$ Å, $\beta = 102.51(3)°$, $V = 2480.8(13)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.153$ g cm^{-1}, $\mu = 0.072$ mm^{-1}, $I > 2\sigma(I)$, $F(000) = 928$, 2θ range 4.0–48.0, 3898 reflections collected, 3258 independent, 402 refined parameters, $\Delta\rho_{max} = 0.243$, $wR = 0.1912$, $R_{obs} = 0.068$.

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Mevalonate-Independent Biosynthesis of Terpenoid Volatiles in Plants: Induced and Constitutive Emission of Volatiles**

Jörn Piel, Jens Donath, Katja Bandemer, and Wilhelm Boland*

Dedicated to Professor Lothar Jaenicke on the occasion of his 75th birthday

Plants utilize volatiles to overcome constraints that result from their stationary way of life. Volatiles may serve as attractants for pollinators[1, 2] or may attract prey to carnivorous plants[3] over long distances. Herbivore-induced plant volatiles can even serve as cues to direct predators into the vicinity of their prey;[4,5] this signaling of the plant to the third trophic level is frequently interpreted as "a plant's cry for help".[6] Emission of volatiles such as ethylene, methyl jasmonate, or methyl salicylate by plants under attack may even induce defense and resistance genes in undamaged neighboring plants.[7–9] For example, in the dicotyledonous lima bean (*Phaseolus lunatus)* volatile emission can be induced by treatment with jasmonic acid or spider mite infestation.[5, 10, 11] As shown in Figure 1, a large proportion of

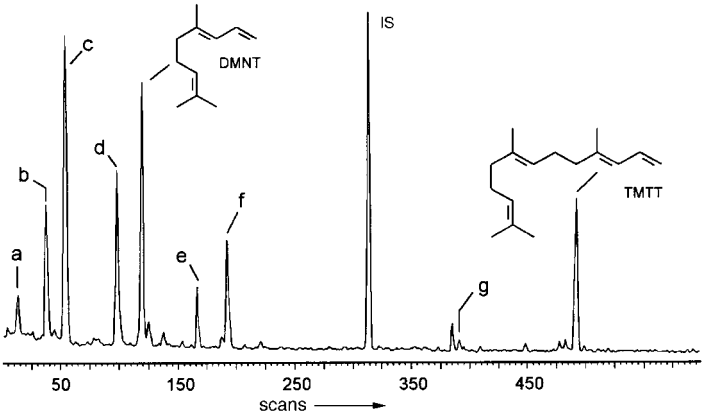

Figure 1. Profile of volatiles emitted from leaves of lima beans (*Phaseolus lunatus*) after infestation with spider mites (for details see Experimental Section). Identified compounds: a: (*Z*)-hex-3-enyl acetate; b: 2-ethylhexanol (contamination); c: ocimene; d: linalool; e: methyl salicylate; f: $C_{10}H_{14}O$; g: geranylacetone; DMNT, TMTT, IS: (1-bromodecane) as internal standard. After induction of lima beans with jasmonic acid (JA) a comparable blend of volatiles is released with exception of TMTT.[10]

the emitted volatiles are terpenoids. Particularly noteworthy are the two homoterpenes 4,8-dimethylnona-1,3,7-triene (DMNT) and 4,8,12-trimethyltrideca-1,3,7,11-triene (TMTT) emitted by many higher plants in response to herbivore attack[12, 13] and which are known to attract insectivores.[4, 5] Both homoterpenes are synthesized de novo: mass spectro-

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metric studies of the volatiles emitted by cotton plants in response to herbivore feeding revealed the incorporation of ^{13}C-labeled CO_2 into several terpenoids.[14] Differences in the extent of labeling of mono- and sesquiterpenoids were also observed after pre-treatment of freshly cut leaves of the lima bean with an aqueous solution of deuterated mevalonate ([D_5]MVA) followed by induction of the biosynthesis of the terpene by jasmonic acid (JA) or by infestation with spider mites.[10, 11] Accordingly, the degree of labeling of [D_8]DMNT was about 88%, whereas monoterpenoids like [D_4]ocimene and [D_4]linalool exhibited a degree of labeling less than 20% (see Figures 1 and 2).[15]

Here we report that the differences in the extent of labeling of individual terpenoids is attributed to a different origin of their respective C_5 precursors from the long known mevalonate-dependent[16] and the recently discovered mevalonate-independent[17–20] pathways that both lead to isopentenyl-pyrophosphate (I-PP), the universal precursor for all terpenoids (see Scheme 1). Furthermore, we show that a parallel utilization of both pathways is possible for inducible and constitutively emitted volatiles from flowers and leaves.

Freshly cut plantlets were placed in a solution containing jasmonic acid and deuterated [D_5]MVA[21] or deuterated deoxy-D-xylulose ([D_2]DOX),[22] an intermediate[23] of the mevalonate-independent route to I-PP, and volatiles were collected after 24 h.[24] For the constitutive emission of volatiles from flowers and leaves no preceding induction by JA is necessary. The incorporation of the two different precursors was monitored by mass spectrometry of the trapped deuterated metabolites. In general, the extent of the labeling of the de novo synthesized compounds was high (see Figure 2). Without exception, the maximum number of isotopes present in the metabolites was in agreement with the numbers of deuterium atoms expected from the utilization of either [D_2]I-PP (from [D_2]DOX) or [D_4]I-PP (from [D_5]MVA). Figure 2 shows that the incorporation of [D_2]DOX was particularly high in the monoterpenes [D_4]ocimene (90%) and [D_4]linalool (90%); the extent of labeling of the sesquiterpene-derived dimethylnonatriene[25] (DMNT, see Scheme 1) was clearly lower (approximately 40% [D_3]DMNT). Instead, highly labeled [D_8]DMNT (up to 88%) was obtained after preincubation with [D_5]MVA. On the other hand, after pretreatment with [D_2]DOX and simultaneous blocking of the MVA route with inhibitors like Mevastatin[26] or Cerivastatin[27] the resulting [D_3]DMNT also showed a high degree of labeling (approximately 70% [D_3]DMNT), indicating that the biosynthesis of this important signaling compound may follow both routes. Unlike DMNT, inhibition of the MVA route could not increase the already high incorporation of [D_2]DOX into the monoterpenes ocimene and linalool, thus, confirming the rather low importance of the MVA pathway for the biosynthesis of monoterpenes (Figure 2).

The incorporation of [D_2]DOX into the diterpene derived TMTT[25] was very low after elicitation with jasmonic acid.[10] Even blocking of the MVA route with Cerivastatin did not significantly enhance the incorporation of [D_2]DOX into this compound (Figure 2). However, if preincubated leaves of the lima bean were infested by spider mites, high amounts of labeled [D_5]TMTT (Figure 1) were emitted (up to 92%).

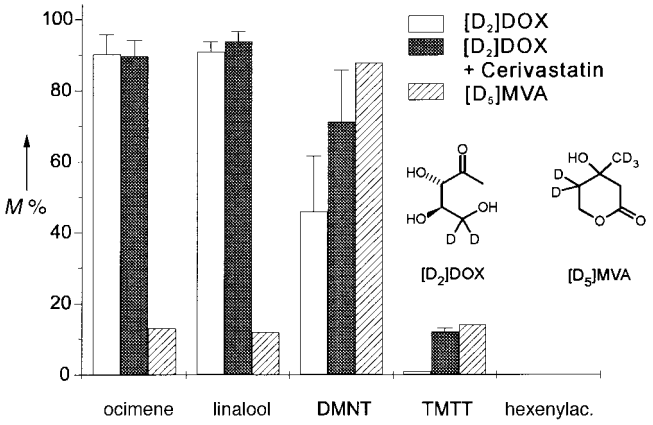

Figure 2. Degree of labeling of induced volatiles (JA treatment or spider mite infection) after preincubation of lima bean leaves (*P. lunatus*) with deuterated deoxy-D-xylulose ([D_2]DOX) or deuterated mevalolactone ([D_5]MVA). Separation and identification of compounds as described for Figure 1. Shown are relative amounts [%] of deuterium-labeled compounds of the total amounts of deuterium-labeled and unlabeled compounds. Incorporation of [D_2]DOX into terpenoid resulted in [D_4]ocimene, [D_4]linalool, [D_3]DMNT, and [D_5]TMTT. Incorporation of [D_5]MVA leads to [D_8]ocimene, [D_8]linalool, [D_8]DMNT, and [D_{12}]TMTT. The fatty acid derived hexenyl acetate showed no incorporation at all. Therefore, a random distribution of isotopes through degradation of [D_2]DOX or [D_5]MVA and reuse of the produced acetyl-CoA does not occur. The degree of labeling M was taken directly from the distribution of the isotopomers of the molecular ions or from the degree of labeling of the terminal prenyl fragments (M_P) ($C_5H_9^+$, $m/z = 69$, unlabeled; $C_5H_7D_2^+$, $m/z = 71$ after incorporation of [D_2]DOX or $C_5H_7D_4^+$, $m/z = 73$ after incorporation of [D_5]MVA). Given a uniform incorporation of (labeled) I-PP into higher terpenes, the degree of labeling was extrapolated according to the equation $M_{DMNT} = 2M_p - M_P^2$ and $M_{TMTT} = 1 - (1 - M_p)^3$. After application of [$D_2$]DOX and spider mite infection labeled [D_5]TMTT (up to 94%) was found. The figure presents only the degree of labeling after JA treatment.

Scheme 1 illustrates the importance of the novel biosynthetic pathway (deoxyxylulose route) for the de novo synthesis of (volatile) terpenoids in lima beans. According to the above findings, [D_2]DOX is preferably channeled into the mono- and diterpene biosynthesis in the plastids. The incorporation of [D_2]DOX into sesquiterpenes (e.g. DMNT as a metabolite of nerolidol) that are commonly synthesized from mevalonic acid in the cytosol, is considerably lower, but not negligible (Figure 2). Apparently, a significant exchange of precursors (e.g. of isopentenyl–pyrophosphate) occurs between the cytosolic and the plastid compartments.[28, 29] This exchange could allow stressed plants to use increasing amounts of plastidic precursors to secure the biosynthesis of sesquiterpenes as phytoalexins, if the MVA route is blocked or insufficient (Scheme 1). Whether this flexible resource management is of general importance to the plant terpene biosynthesis, or whether a deoxyxylulose route exists in the cytosol, remains to be explored.

The above findings are not limited to the dicotyledonous lima bean. High levels of incorporation of [D_2]DOX in JA-induced terpenes were also observed for tobacco (*Nicotiana plumbaginifolia*) and the monocotyledonous maize *(Zea mays)*. After stimulation with JA, maize showed high levels of labeling of both homoterpenes ([D_3]DMNT, 80%;

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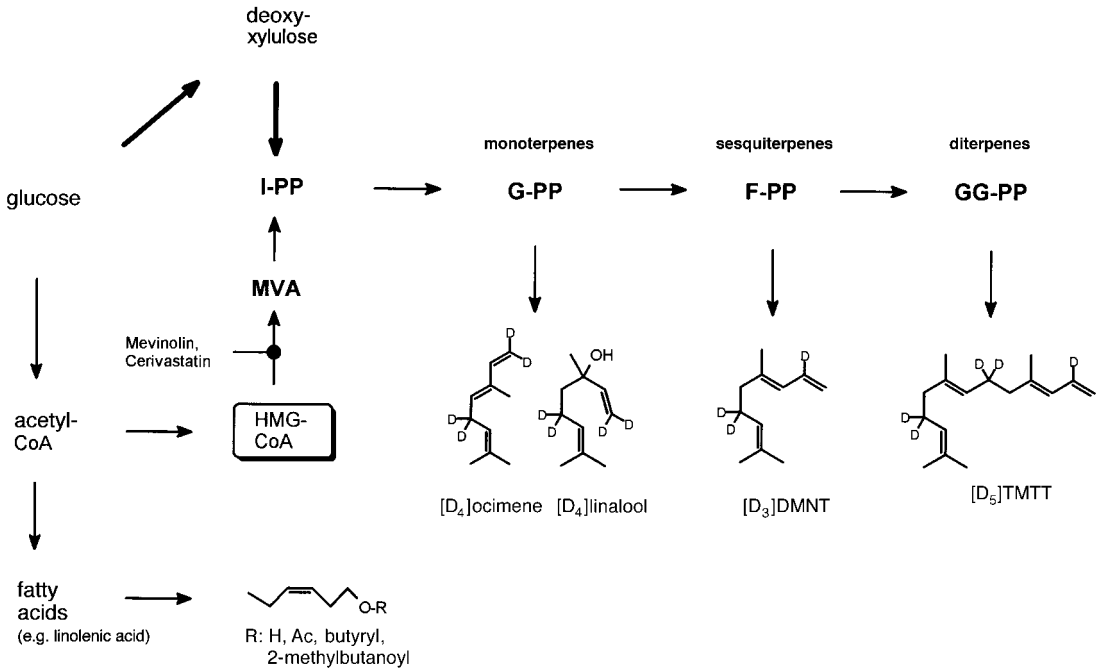

Scheme 1. Survey of inducible volatile production in lima beans, along the alternative pathways of biosynthesis. The mevalonate-independent route can be blocked by inhibitors of the HMGCoA-reductase (Mevinolin, Cerivastatin). The different labeling patterns of volatile terpenes after incubation with [D_2]DOX are shown; the expected positions of the deuterium isotopes according to the rules for terpene biosynthesis were confirmed by mass spectrometric analysis. I-PP: isopentenyl diphosphate, G-PP: geranyl diphosphate, F-PP: farnesyl diphosphate, GG-PP: geranylgeranyl diphosphate.

Table 1. Degree of labeling *M* (determined mass spectrometrically from the masses of the molecular ions) of constitutively emitted leaf and flower volatiles after incubation with [D_2]DOX.

Eucalyptus globulus (leaf)		*Clematis vitisalba* (flower)		*Hedera helix* (flower)		*Passiflora caerulea* (flower)		*Callicarpa japonica* (flower)	
compound	*M* [%]	compound	*M* [%]	compound	*M* [%]	compound	*M* [%]	compound	*M* [%]
1,8-cineole	0	β-pinene	30	sabinene	25	ocimene	83	β-pinene	74
limonene	0	limonene	24	β-myrcene	36	β-citronellal	19	linalool	20
ocimene	83	ocimene	77	limonene	20	geranylacetone	45	β-citronellol	12
DMNT	95	oxoisophorone[a]	0	oxoisophorone[a]	0	farnesene	81	nerol	37
caryophyllene	83	dihydrooxoisophorone[a]	0	dihydrooxoisophoronе[a]	0	TMTT	95		
nerolidol	94	*endo*-borneol	15	linalool	83				
TMTT	99	nerol	33	linalooloxide	50				
		α-terpineol	11	farnesene	89				
		α-verbenone	14						
		caryophyllene	70						

[a] Compounds that will lose any incorporated deuterium during the biosynthesis of oxo- and dehydrooxoisophorone.

[D_5]TMTT, 96%). Even in the evolutionary old gingko tree (*Gingko biloba*) biosynthesis of JA-inducible sesquiterpenes like linalool, copaene, caryophyllene, and farnesene mostly utilizes [D_2]DOX.

Similar observations were made upon treatment of flowers or leaves that constitutively emit volatiles with [D_2]DOX. Thus, volatile terpenoids from [D_2]DOX-treated flowers of *Passiflora caerulea*, *Clematis vitalba*, *Hedera helix, Callicarpa japonica* and from leaves of *Eucalyptus globulus* were collected and analyzed. The results are compiled in Table 1. Depending on their different individual metabolic history (storage in vacuoles, release from conjugates, mevalonate-dependent biosynthesis) the degree of labeling of single compounds varied considerably (0 to 100%).

The results presented here demonstrate that the recently discovered mevalonate-independent pathway (deoxyxylulose route) is the major pathway towards volatile terpenoids in plants including the light-dependent release of isoprene from leaves (willow, poplar, etc.).[19] However, the most important result of this work is the massive incorporation of [D_2]DOX into sesquiterpenes and the flexible management of resources between cytosol and chloroplast that allows for a well-adjusted response of plants to stresses like herbivore- or pathogen infestation.

Experimental Section

For experiments with lima beans, 11–15-day-old seedlings with two fully developed primary leaves were cut and immediately placed into vials containing a solution of the deuterium-labeled precursors ([D_5]MVA: 3.0 mg mL^{-1}, [D_2]DOX. 2.0 mg mL^{-1}) in tap water; the damage caused by cutting of the stem did not induce volatile emission. After 48 h preincubation under light (270 μE m^{2}s^{-1}), JA was added and adjusted to a final

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concentration of 10.0 $\mu g\,mL^{-1}$; alternatively pretreated leaves were infested with spider mites (*Tetranychus urticae*, approximately 50 mites per leaf; source: Neudorf, D-31860 Emmerthal, Germany) to induce volatile production. The vials with the leaves were placed into a closed system consisting of a desiccator (750 mL) equipped with a miniature circulation pump, fitted to a trap with activated charcoal (1.5 mg) for collection of the volatiles from the circulating air stream.[24] The collection of volatiles was maintained for 24 h and then the compounds were desorbed from the carbon traps with CH_2Cl_2 (2×20 µL) and immediately analyzed by GLC-MS. Inhibitor experiments with Mevastatin or Cerivastatin were conducted by sequential treatment of the plants with the inhibitor for 24 h (Mevastatin: 0.2–0.5 $mg\,mL^{-1}$; Cerivastatin: 0.2 $mg\,mL^{-1}$) followed by incubation with [D_5]MVA or [D_2]DOX and an elicitor as described above. All experiments were repeated at least twice ($n = 3$) to secure reproducibility. The GC-MS analyses were performed on a Fisons MD 800 equipped with a fused silica column, coated with SE 30 (15 m × 0.31 mm). The temperature was programmed from 50 °C (2 min isothermal) to 200 °C at 10 °C min^{-1}; GC-interface: 270 °C; scan area 35–350 $Da\,s^{-1}$.

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Catalytic Enantioselective Retro-Aldol Reactions: Kinetic Resolution of β-Hydroxyketones with Aldolase Antibodies**

Guofu Zhong, Doron Shabat, Benjamin List, James Anderson, Subhash C. Sinha, Richard A. Lerner,* and Carlos F. Barbas III*

The development of methodologies for the stereoselective synthesis of β-hydroxyketones (aldols) has long been the subject of intensive studies in organic chemistry. This challenge was initially met through the use of stoichiometric amounts of chiral auxiliaries[1] and most recently by the design of transition metal based catalysts.[2] An alternative to direct enantioselective synthesis is the application of the strategy of kinetic resolution.[3, 4] Surprisingly, the application of this strategy to the aldol problem has received little, if any, attention.[3b, 5, 6] Recently we have described the generation, broad scope, structure, high stereoselectivity, and mechanism of two aldolase antibodies 38C2 (Aldrich: Aldolase antibody 38C2, cat. no. 47,995-0 and 48,157-2) and 33F12.[7] Since these antibodies are able to catalyze both the aldol addition and the retro-aldol reaction we envisioned that they may be useful in the kinetic resolution of aldols. A single antibody catalyst could then be used for the preparation of both aldol enantiomers (Scheme 1).

In order to address the potential of antibody-catalyzed kinetic resolution of aldols, we studied the kinetics of the retro-aldolization of (*S*)- and (*R*)-**1**. Antibody 38C2 catalyzed the retro-aldolization of (*S*)-**1** following Michaelis–Menten kinetics ($k_{cat} = 1.4\ min^{-1}$, $K_m = 270\ \mu M$). The relative rate

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