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The stereochemical course of tricho-acorenol biosynthesis†

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The biosynthesis of tricho-acorenol in *Trichoderma harzianum* was investigated by feeding stereospecifically deuterated mevalonolactone isotopomers, followed by a detailed GC/MS analysis of the incorporation of labelling. The results establish a highly stereospecific hydride migration and antarafacial attack of water in the terminal step towards tricho-acorenol.

Fungi of the genus *Trichoderma* are well known to be plant growth promoting symbionts. Due to their biological activity against phytopathogenic bacteria, *Trichoderma* species are used as biocontrol agents in agriculture.[1,2] Several natural products are known from *Trichoderma* such as the cytotoxic trichodermamides[3] and harziphilone,[4] the mycotoxin T-2 toxin,[5] and a series of volatile pyrones[6–9] and acorane sesquiterpenes[10,11] of unknown functions. One of the strongest producers of volatile terpenes, *T. harzianum*, showed the production of large quantities of the sesquiterpenes tricho-acorenol **1** and acorenone **2** (Fig. 1).[11] The biosynthesis of these compounds was investigated in feeding experiments with deuterated mevalonolactone isotopomers[12] and proceeds *via* several cyclisation steps in combination with three hydride shifts and water capture to **1**, while **2** requires one additional oxidation step (Scheme 1).[11]

In detail, the biosynthesis of **1** starts with the rearrangement of farnesyl diphosphate **3** by extrusion and reattack of the diphosphate moiety to nerolidyl diphosphate **4**. Its reionisation initiates the first cyclisation to the bisabolyl cation **5**. After a 1,2-hydride shift to the homobisabolyl cation **6** a second cyclisation affords the acorenyl cation **7**. As demonstrated in previous experiments with deuterated mevalonolactones the following steps include a combination of a 1,4- and a 1,2-hydride shift *via* cation **8** to **9** that is trapped by water to yield **1**.[11] The third hydride shift in this biosynthetic scheme from **8** to **9** can occur suprafacially on either face of the six-membered ring, followed by a final attack of water at C-3 that may proceed supra- or antarafacially to the migrating hydride. The stereochemical course of these late steps was investigated by feeding of stereospecifically deuterated mevalonolactone

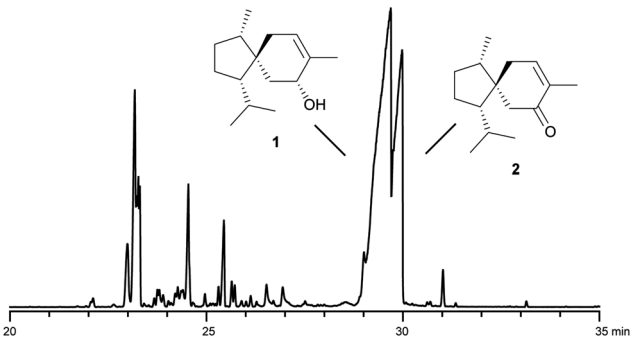
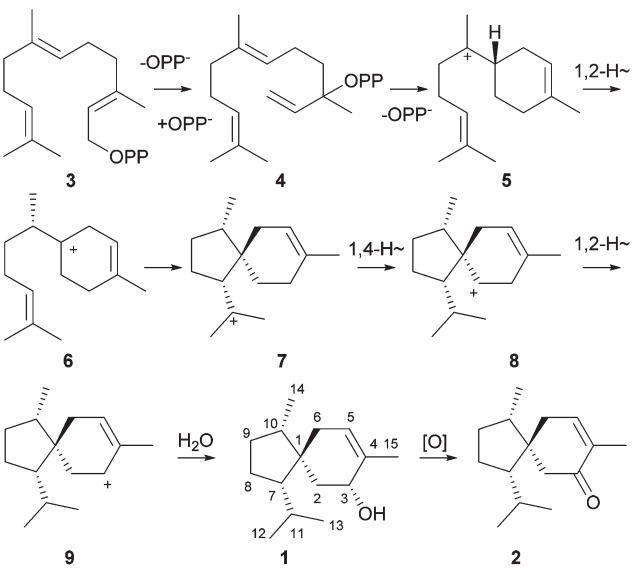


Fig. 1 Gas chromatogram of the headspace extract of *T. harzianum*.



Scheme 1 Biosynthesis of **1** and **2** in *T. harzianum*.

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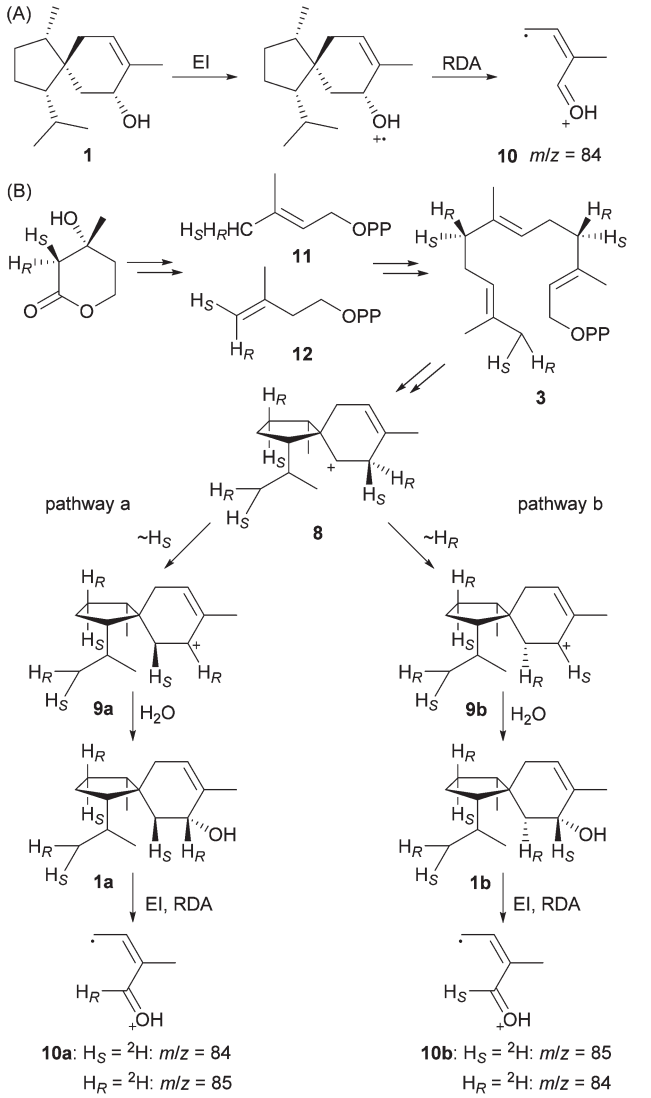
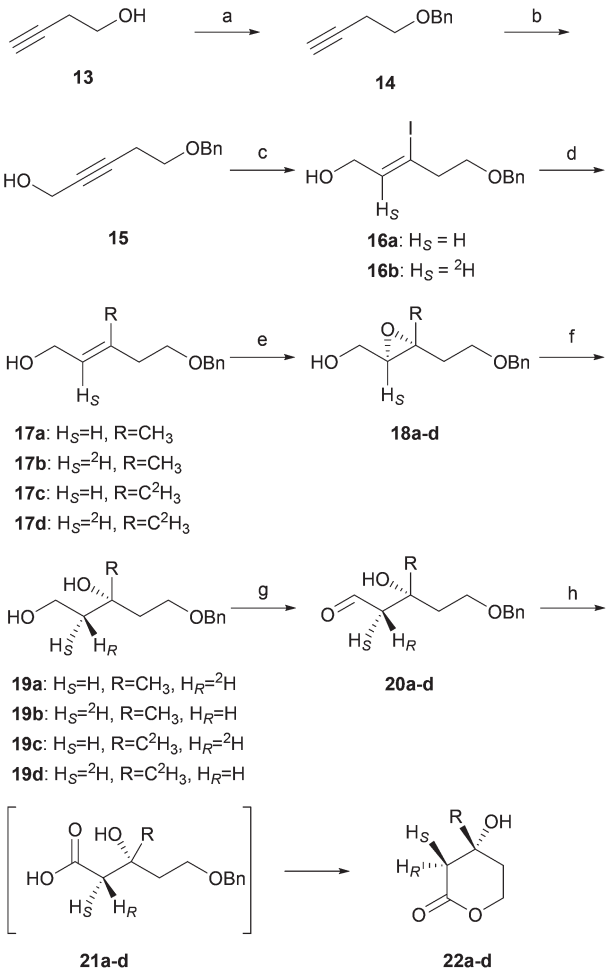


Scheme 2 (A) Retro-Diels-Alder fragmentation of **1** in EI mass spectrometry; (B) possible outcomes of feeding experiments with stereospecifically deuterated mevalonolactone isotopomers.



Scheme 3 Synthesis of deuterated mevalonolactone isotopomers. (a) NaH, BnBr, 92%; (b) *n*-BuLi, $(CH_2O)_n$, 67%; (c) Red-Al (or $LiAl^2H_4$), NIS, 92%, (65%); (d) Me_2CuLi or $[^2H_6]Me_2CuLi$, 82–86%; (e) $Ti(OiPr)_4$, (–)-(D)-DIPT, 84–88%; (f) $LiAlH_4$ or $LiAl^2H_4$, 86–90%; (g) IBX, 86–87%; (h) 1. $NaClO_2$, 2. H_2, Pd/C, 45–83% (2 steps).

isotopomers, and the results of these investigations are reported here.

The biosynthesis of **1** was investigated by feeding of deuterated mevalonolactone isotopomers. Incorporation of labelling was followed by capturing the volatiles on charcoal using a closed-loop stripping apparatus (CLSA) and GC/MS analysis of the obtained headspace extracts. The EI mass spectrum of **1** exhibits a major fragment ion at m/z = 84 that arises from Retro-Diels Alder (RDA) fragmentation (Scheme 2A). This fragment ion can be used as a probe for the stereochemical problem under investigation. During FPP cyclisation to **1** either the original 2-*pro-R* or the 2-*pro-S* hydrogen of mevalonolactone, ending up in stereochemically defined positions of **3**[13,14] and at C-3 of cation **8**, is shifted to the adjacent C-2 in **9** (Scheme 2B). Deuterated mevalonolactones with specific labelling in the 2-*pro-R* and 2-*pro-S* positions were used, leading to diagnostic fragment ions at m/z = 84 or m/z = 85, to distinguish between two alternative stereochemical courses (pathways a and b in Scheme 2B).

For this purpose the mevalonolactones **22a–d** were synthesised *via* known **17**[15] (Scheme 3). Homopropargyl alcohol **13** was protected as benzyl ether **14** that was deprotonated with *n*-BuLi followed by reaction with *para*-formaldehyde to yield alcohol **15**. Reduction of the alkyne with Red-Al and quenching with *N*-iodosuccinimide gave the vinyl iodide **16a**. When Red-Al was replaced with $LiAlD_4$ deuterated **16b** was obtained. The alkenyl iodides **16** were alkylated with Me_2CuLi resulting in **17a** and **17b**. Alternatively, deuterated $[^2H_6]$-Me_2CuLi was used to yield the isotopomers **17c** and **17d**. Sharpless epoxidation resulted in the epoxides **18a–d** with 95% ee based on Mosher analysis[16] (reported ee: 94%).[17] Reductive opening of the epoxide with $LiAlH_4$ or $LiAlD_4$ gave the diols **19** with stereospecific labelling. The diols were oxidised to the aldehydes **20** followed by Pinnick oxidation to the acids **21** that were used in the next step without purification. Finally, hydrogenolysis of the benzyl protecting group afforded

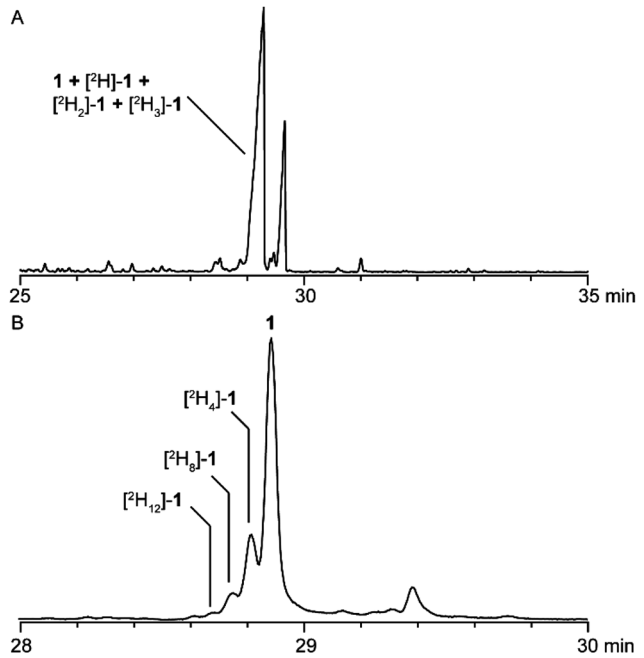


Fig. 2 Total ion chromatograms of the headspace extracts from *Trichoderma harzianum*. (A) Total ion chromatogram of a headspace extract obtained in the feeding experiment with **22b**; (B) feeding experiment with **22d**. Comparable results were obtained in the feeding experiments with **22a** and **22c**, respectively.

the mevalonolactones **22** in high yields of 8–32% (9 steps, based on the minimum and maximum yields for each step).

The synthetic compounds were fed to agar plate cultures of *T. harzianum*. The emitted volatiles were collected using the CLSA technique[18] and the obtained headspace extracts were analysed by GC/MS. It is well known that deuterated compounds can be gas chromatographically separated from their non-deuterated analogs,[19] and this separation is better the higher the deuterium content is. This is one of the major advantages of the GC/MS technique in analysing the incorporation of deuterium labelled precursors into volatile natural products, because a "clean" mass spectrum of the deuterated compound that is not superimposed with the mass spectrum of the unlabelled analog can be obtained, allowing for a detailed analysis. Although feeding of **22a** and **22b** resulted in the incorporation of up to three deuterated isoprene units into **1** (Fig. 2A), the low differences in the deuterium content of the various isotopomers of **1** were insufficient for a gas chromatographic separation which prohibited unambiguous data interpretation. To circumvent this problem the mevalonolactones **22c** and **22d** with an additional deuterium labelling in the methyl groups were synthesised and fed to the fungus. The higher deuterium content of four deuterium atoms per isoprene unit resulted in a clear separation of the isotopomers of **1** in GC according to their deuterium content (Fig. 2B), allowing for an accurate data interpretation.

The resulting mass spectra from the feeding experiments with **22c** and **22d** are presented in Fig. 3. The mass spectrum of natural **1** shows a molecular ion at m/z = 222 (Fig. 3A). After

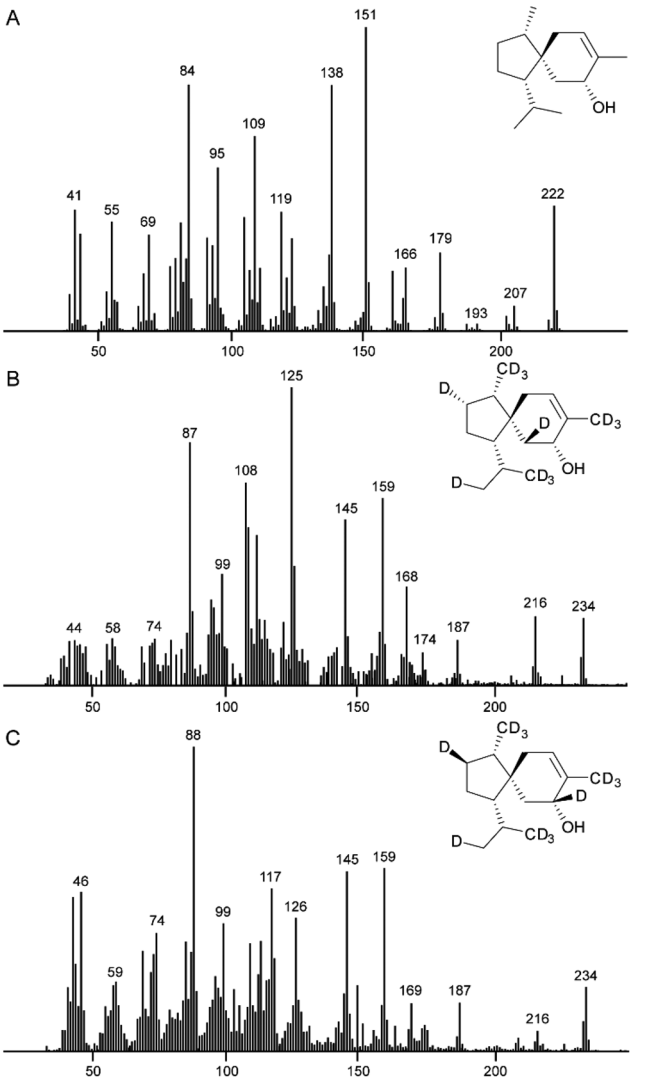


Fig. 3 Mass spectra of **1**. (A) Mass spectrum of authentic **1**;[20] (B) mass spectrum of [$^2H_{12}$]-**1** obtained after feeding of **22d**; (C) mass spectrum of [$^2H_{12}$]-**1** obtained after feeding of **22c**.

feeding of **22d** (H_S = ^{2}H, R = C^{2}H$_3$) the molecular ion for the isotopomer with the highest deuterium content of **1** is shifted to m/z = 234, indicating that up to three isoprene units each carrying four deuterium atoms were incorporated (Fig. 3B). The diagnostic fragment ion at m/z = 84 is shifted to m/z = 87 due to the incorporation of three deuterium atoms. This finding is in agreement with pathway a, but not with pathway b in Scheme 2. In a complementary experiment, **22c** (H_R = ^{2}H, R = C^{2}H$_3$) was fed to *T. harzianum*, also resulting in the incorporation of up to twelve deuterium atoms (Fig. 3C). The diagnostic Retro-Diels-Alder fragment ion is observed at m/z = 88, again in full agreement with pathway a and excluding pathway b of Scheme 2. These observations clearly demonstrate that the final of a series of three hydride shifts during the biosynthesis of **1** proceeds stereospecifically with migration of the original 2-*pro-S* hydrogen (H_S) of mevalonolactone. The subsequent nucleophilic attack of water at the cationic centre

occurs antarafacially at C-3 with respect to the migrating hydride.

Conclusions

In summary the stereochemical course of the final hydride migration in tricho-acorenol biosynthesis was followed by feeding experiments with stereospecifically deuterated mevalonolactone isotopomers that were obtained by chemical synthesis. Careful analysis of the incorporation of isotope labellings by GC/MS established that the final 1,2-hydride migration proceeds specifically with shifting of the original 2-*pro-S* hydrogen (H_S) from mevalonolactone, followed by a nucleophilic attack of water at C-3 in an antarafacial manner with regard to the migrating hydride. Further mechanistic studies on terpene biosynthetic pathways are now possible to gain a deeper understanding of terpene cyclases as some of the most fascinating enzymes in natural product biosynthesis.

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