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Geosmin Biosynthesis. Mechanism of the Fragmentation–Rearrangement in the Conversion of Germacradienol to Geosmin

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(−)-Geosmin (**1**) is a degraded sesquiterpene that is responsible for the characteristic odor of moist soil and is associated with unpleasant off-flavors in water, wine and fish.[1] Geosmin is produced by a number of microorganisms, including most *Streptomyces* and several species of cyanobacteria, myxobacteria and fungi.[2]

A single 726-amino acid protein in *Streptomyces coelicolor* A3(2)catalyzes the Mg^{2+}-dependent cyclization of conversion of farnesyl diphosphate (**2**, FPP) to a mixture of germacradienol (**3**), germacrene D (**4**), and geosmin (**1**),[3,4] accompanied by small amounts of octalin **5**.[5] The closely related 725-amino acid GeoA protein of *S. avermitilis* with 78% identity and 85% similarity to the *S. coelicolor* enzyme catalyzes the identical reaction.[6] The *S. coelicolor* germacradienol/geosmin synthase is a bifunctional enzyme in which the N-terminal domain of the protein converts FPP (**2**) to germacradienol (**3**) and **4**, while the C-terminal domain catalyzes the transformation of germacradienol (**3**) to geosmin (**1**).[7] Both the N-terminal and C-terminal halves have significant sequence similarity to the well-characterized sesquiterpene synthase, pentalenene synthase.[3a,7,8]

The mechanism and stereochemistry of the conversion of FPP to **3** and **4**, which is thought to involve the partitioning of a common germacradienyl cation intermediate **6**, has been investigated in detail (Scheme 1a).[3,4,6,7] Formation of germacrene D (**4**) results from a 1,3-hydride shift of the original H-1*si* of FPP.[3b] The alternative formation of germacradienol (**3**), which involves competing loss of the H-1*si* proton of FPP (**2**), can occur by cyclization of **6** to an enzyme-bound, *trans*-fused bicyclic intermediate, isolepidozene (**7**), a compound that has been isolated from incubation of FPP with the S233A mutant of *S. coelicolor* germacradienol/geosmin synthase.[7] Isolepidozene (**7**) would be converted to germacradienol (**3**) by proton-initiated ring opening and capture of the resulting homoallyl cation by water.[4,7]

By contrast, the mechanistic details of the subsequent conversion of germacradienol (**3**) to geosmin (**1**) are still incomplete. Independent incorporation experiments with labeled mevalonates using *Myxococcus xanthus* and *Stigmatella aurantiaca* support the mechanism of Scheme 1a in which proton-initiated cyclization of germacradienol and retro-Prins fragmentation result in formation of octalin **5** and release of the 2-propanol side chain as acetone (**8**).[9] Reprotonation of **5** followed by 1,2-hydride shift of the bridgehead proton into ring B and quenching of the resulting cation by water will generate geosmin (**1**).[9] This model is supported by the isolation of octalin **5** as a coproduct of incubations of FPP with germacradienol/geosmin synthase.[5–7] By contrast, an alternative 1,2-hydride shift of the same bridgehead hydrogen into ring A of geosmin during biosynthesis in the liverwort *Fossombronia pusilla* has also been proposed, based on incorporations of labeled mevalonate.[10] It has been suggested that this mechanism is also operative in *Streptomyces* sp. JP95 (Scheme 1b).[10] We

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now report evidence that conversion of germacradienol (**3**) to geosmin (**1**) by *S. coelicolor* germacradienol/geosmin synthase results in the release of the three-carbon side chain as acetone and involves a 1,2-hydride shift of the bridgehead hydrogen exclusively into ring B of geosmin.

To detect acetone generated in the formation of geosmin, the product mixture from incubation of FPP with recombinant *S. coelicolor* germacradienol/geosmin synthase was reacted with cysteamine (Scheme 2a).[11] GC-MS analysis confirmed the formation of 2,2-dimethylthiazolidine (**9**) which displayed a parent peak at *m/z* 117 and a prominent [M-CH_3]$^+$ at *m/z* 102.. Control experiments established that neither geosmin nor acetone was formed when the protein was first inactivated by boiling. To confirm the origin of the enzymatically generated acetone, [13,13,13-$^2H_3$]FPP (**2a**)[12] was incubated with germacradienol/geosmin synthase. The [$^2H_3$-Me]-2,2-dimethylthiazolidine (**9a**) derived from the resulting deuterated acetone showed a molecular ion at *m/z* = 120 [M+3]$^+$ with fragment ions at *m/z* = 102 and 105 resulting from loss of the CD_3- and CH_3- groups, respectively (Figure 1). The presence of the trideuterated 2-hydroxypropyl moiety in the intermediate [12,12,12-$^2H_3$]-germacradienol (**3a**) was indicated by a shift of the molecular ion [d_3-M]$^+$ from *m/z* = 222 to 225 and a corresponding shift in the base peak from *m/z* = 59 to 62 [$CH_3(CD_3)$ C=OH]$^+$, while the [M-acetone]$^+$ fragment at *m/z* 164 was unchanged. The mass spectrum of the [12,12,12-$^2H_3$]germacrene D (**4a**) co-product also displayed all the predicted changes. The mass spectra of the derived geosmin (**1**, *m/z* 182) and octalin (**5**, *m/z* 164) confirmed the complete absence of deuterium label in either of these C_{12} products.

To explore the fate of the H-2 proton of FPP, the requisite [2-^{2}H]FPP (**2b**) (>99 atom% deuterium) was synthesized from trideuteroacetic acid by way of [2,2-$^2H_2$]trimethylsilylacetic acid using a modified Peterson olefination procedure that avoids exchange of the deuterium label.[13] GC-MS analysis of the products resulting from cyclization of [2-^{2}H]FPP (**2b**) showed the predicted germacradienol-d_1 (**3b**), germacrene D-d_1 (**4b**), octalin-d_1 (**5b**) and geosmin-d_1 (**1b**) (Scheme 2b). In the mass spectrum of unlabeled geosmin, besides the weak molecular ion (*m/z* = 182), two other well-defined fragments at *m/z* = 112 and *m/z* = 126 correspond to the parent rings A and B (Figure 2).[9,10] Cyclization of [2-^{2}H]FPP (**2b**) is predicted to generate [6-^{2}H]geosmin (**1b**). The observed site of deuterium labeling in **1b** is consistent with the observed shift from *m/z* 112 to 113 of the characteristic ring B fragment ion; while the corresponding ring A-derived fragment ion from **1b**, *m/z* 126, was devoid of deuterium (Figure 2a). Most importantly, the mass spectrum of **1b** was indistinguishable from that of [6-^{2}H] geosmin derived from (1*R*)-[1-^{2}H]FPP, which should differ from **1b** only in the configuration of the C-6 deuterium (Figure 2b).[4]

The results of conversion of both [13,13,13-$^2H_3$]FPP (**2a**) and [2-^{2}H]FPP (**2b**) to geosmins **1** and **1b** are fully consistent with the proposed mechanism of cyclization and fragmentation of germacradienol (**3**) (Scheme 1a)[4,9] while firmly excluding the mechanism of Scheme 1b[10] as well as alternative, mechanistically less likely proposals.[2b] The Retro-Prins fragmentation that results in the loss of the germacradienol side chain as acetone has no biochemical precedent. There is an exceptionally high level of amino acid sequence conservation (45–78% identity, 57–85% similarity) among more than a dozen known or presumed microbial geosmin synthases.[7] The existence of two independent geosmin biosynthetic pathways, at least among microorganisms, is therefore highly unlikely.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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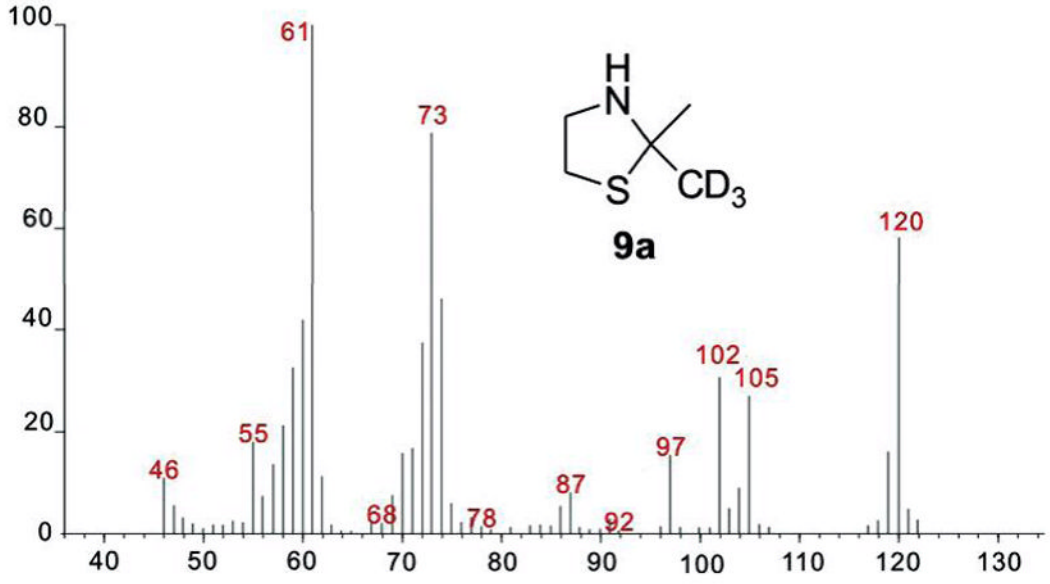


Figure 1.
Mass spectrum of [$^2H_3$-Me]-2,2-dimethylthiazolidine (**9a**).

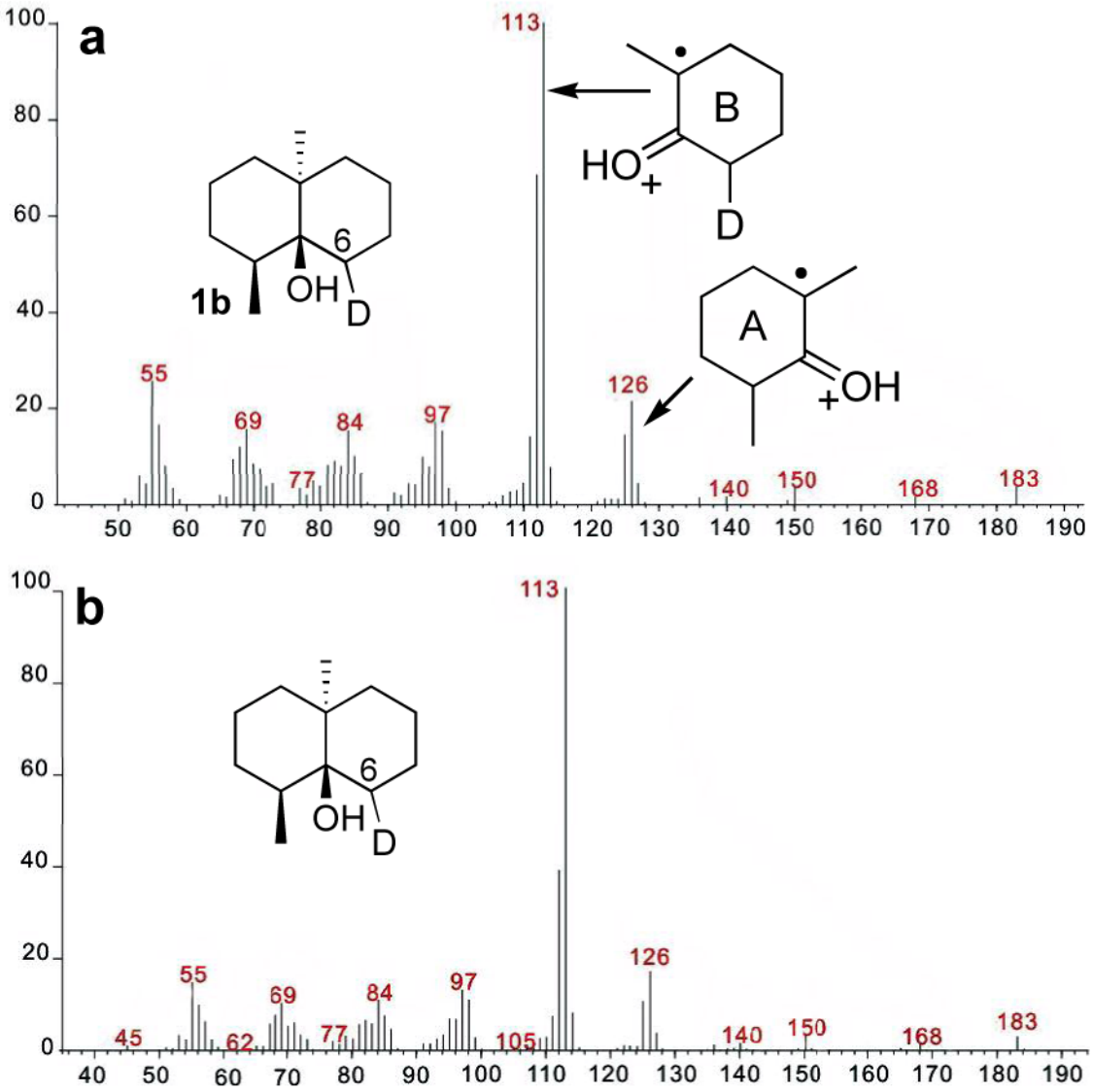
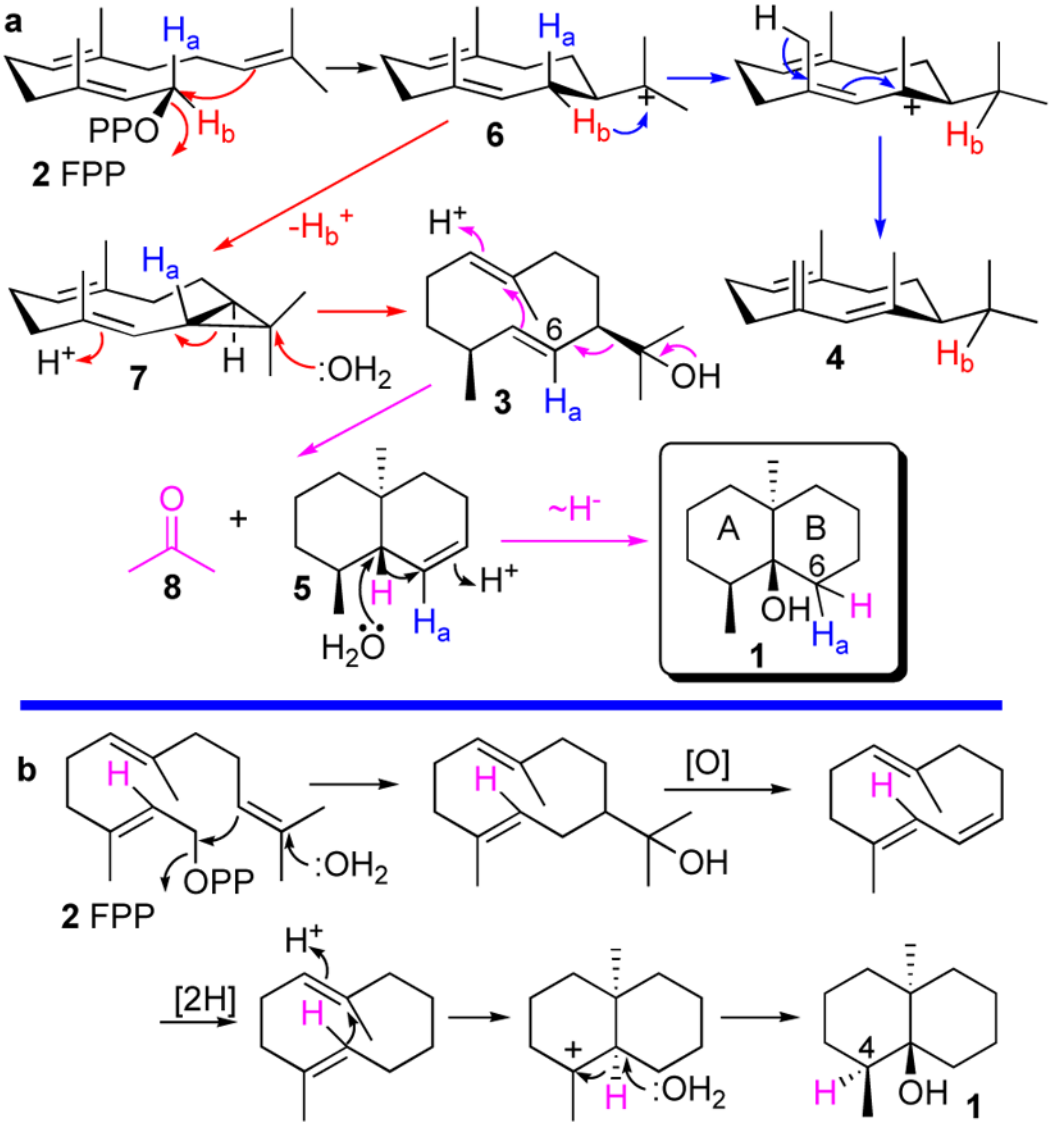
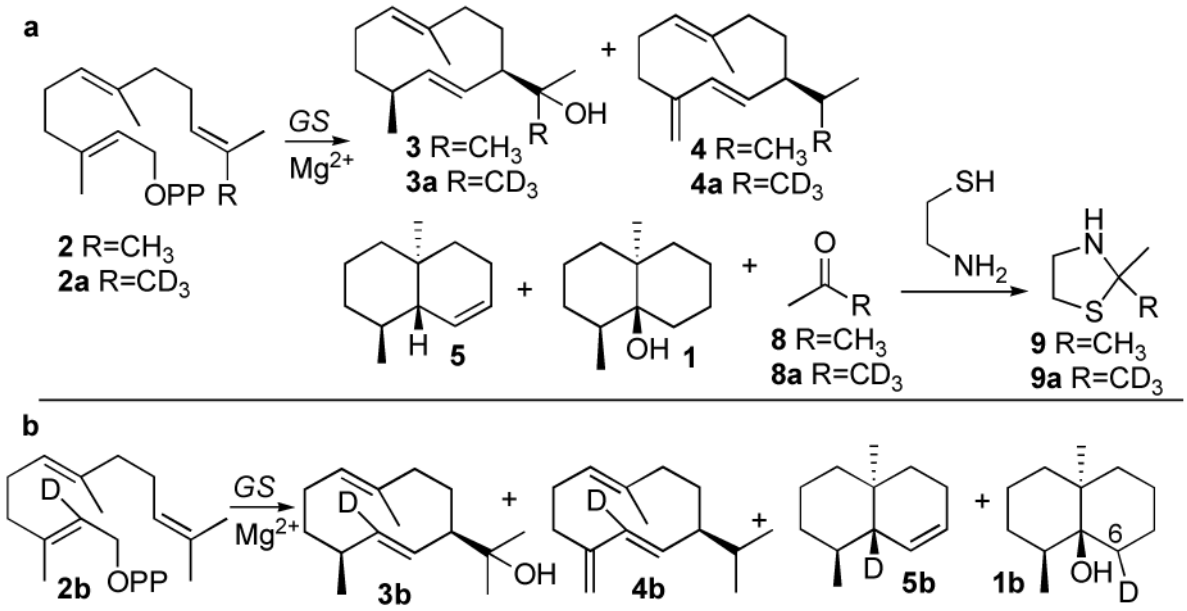


Figure 2.
Mass spectra of [6-^{2}H]geosmin (**1b**) derived from a) [2-^{2}H]FPP and b) (1*R*)-[1-^{2}H]FPP.



Scheme 1.
Cyclization of farnesyl diphosphate to geosmin



Scheme 2.
Cyclization/fragmentation of deuterated FPPs to geosmin